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Omics sciences in the personalization of diagnosis and therapy

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Omics sciences and precision medicine in Urothelial Carcinoma

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Abstract

This comprehensive review explores the potential of omics sciences— such as genomics, transcriptomics, proteomics, and metabolomics—in advancing the diagnosis and therapy of urothelial carcinoma (UC), a prevalent and heterogeneous cancer affecting the urinary tract. The article emphasizes the significant advancements in understanding the molecular mechanisms underlying UC development and progression, obtained through the application of omics approaches. Genomic studies have identified recurrent genetic alterations in UC, while transcriptomic analyses have revealed distinct gene expression profiles associated with different UC subtypes. Proteomic investigations have recognized protein biomarkers with diagnostic and prognostic potential, and metabolomic profiling has found metabolic alterations that are specific to UC. The integration of multi-omics data holds promises in refining UC subtyping, identifying therapeutic targets, and predicting treatment response. However, challenges like the standardization of omics technologies, validation of biomarkers, and ethical considerations need to be addressed to successfully translate these findings into clinical practice. Omics sciences offer tremendous potential in revolutionizing the diagnosis and therapy of UC, enabling more precise diagnostic methods, prognostic evaluations, and personalized treatment selection for UC patients. Future research efforts should focus on overcoming these challenges and translating omics discoveries into meaningful clinical applications to improve outcomes for UC patients. ***Clin Ter 2023; 174 Suppl. 2 (6):1-10 doi: 10.7417/CT.2023.2466***

Key words: *Urothelial Carcinoma, precision medicine, genomics, biomarkers, metabolomics*

Introduction

Urothelial carcinoma is a type of cancer that arises from the cells lining the urinary system, including the bladder, ureters, and renal pelvis (1). It is a significant public health concern, with an estimated 430,000 new cases and 165,000 deaths worldwide in 2020 (2). In the United States alone, approximately 83,000 new cases of bladder cancer are diagnosed annually, with over 17,000 deaths (3). It is more prevalent in men than women, and the risk of developing this disease increases with age (4).

Urothelial carcinoma is a complex and heterogeneous disease that can present in non-invasive or invasive forms, with varying clinical and molecular features (5). Non-invasive urothelial carcinoma, also known as papillary urothelial neoplasms of low malignant potential (PUNLMP) or non-invasive papillary urothelial carcinoma (NIPUC), accounts for approximately 70% of bladder cancers (6). It is typically slow-growing and has a good prognosis, with a low risk of progression to invasive disease. Invasive urothelial carcinoma, on the other hand, can spread to other organs and tissues, leading to metastasis and poor prognoses.

Despite advances in diagnosis and treatment, urothelial carcinoma, also known as transitional cell carcinoma, continues to have poor prognosis, especially in advanced stages (7). This type of cancer is the most common form of bladder cancer and arises from the urothelial cells lining the urinary tract. The current treatment options include surgery, chemotherapy, radiation therapy, and immunotherapy (8). However, the effectiveness of these therapies varies depending on the stage and molecular profile of the tumor (9). To improve patient outcomes, there is an urgent need

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to identify novel biomarkers and therapeutic targets specific to urothelial carcinoma (10). These markers and targets could potentially help in the development of personalized treatment strategies and improve response rates (11). This is particularly important considering the heterogeneity of urothelial carcinoma and the diverse molecular alterations observed in different patients (12).

Omics sciences, including genomics, proteomics, metabolomics, and microbiomics, have revolutionized our understanding of cancer biology and have opened new avenues for the diagnosis, prognosis, and treatment of various cancers, including urothelial carcinoma (13). These omics approaches involve comprehensive analysis of molecular data, encompassing DNA, RNA, proteins, metabolites, and their interactions with the environment and microbiome (14). Genomics, proteomics and metabolomics studies have identified recurrent genetic alterations that drive urothelial carcinoma growth and survival (15). By understanding the altered metabolism in cancer cells, researchers can develop targeted therapies to disrupt these pathways and inhibit tumor progression. In addition to the tumor cells themselves, the role of the microbiome in cancer development and treatment response is being increasingly recognized. Microbiomics has revealed associations between specific microbial compositions and urothelial carcinoma (16). Understanding the interactions between the microbiome and tumor cells can provide insights into the mechanisms underlying carcinogenesis and potentially lead to the development of microbiome-based interventions for urothelial carcinoma.

In this review, we have discussed the use of omics sciences in refining the diagnosis and therapy of urothelial carcinoma. Specifically, we focused on the application of genomics, proteomics, metabolomics, and microbiomics in the diagnosis, prognosis, and treatment of urothelial carcinoma. We propose future directions involving the integration of multi-omics data, the development of novel therapeutic strategies, and the implementation of personalized approaches to cancer management. Omics sciences offer potential in identifying biomarkers, therapeutic targets, and advancing our knowledge of cancer biology in urothelial carcinoma. However, to validate their clinical utility and overcome challenges for widespread adoption, it is crucial to conduct standardized assays, perform biomarker validation, and conduct large-scale studies in clinical practice.

Genetics of cancer

Cancer is a genetic disease that arises from the accumulation of genetic alterations in cells that disrupt normal cellular functions, including cell proliferation, differentiation, and apoptosis. Germline mutations that predispose to cancer, such as mutations in the BRCA1/2 genes, have been extensively studied in breast and ovarian cancer, but their role in urothelial carcinoma is less clear (17). Somatic mutations in oncogenes and tumor suppressor genes play a crucial role in the development and progression of urothelial carcinoma (18). These genetic alterations contribute to the dysregulation of key signaling pathways involved in cell growth, proliferation, and survival. Understanding the role of specific genes in urothelial carcinoma can provide insights into the underlying molecular mechanisms and potential therapeutic targets.

One of the most frequently mutated genes in urothelial carcinoma is fibroblast growth factor receptor 3 (or FGFR3): its mutations are predominantly observed in low-grade non-invasive urothelial carcinomas, including papillary urothelial neoplasms of low malignant potential (PUNLMP) and non-invasive papillary urothelial carcinoma (NIPUC). These mutations lead to constitutive activation of the FGFR3 signaling pathway, promoting cell proliferation and inhibiting differentiation (19, 20). Targeting FGFR3 signaling has emerged as a potential therapeutic strategy for urothelial carcinoma, with the development of FGFR inhibitors currently under investigation in clinical trials (19).

Another commonly mutated gene in urothelial carcinoma is TP53, which encodes the p53 tumor suppressor protein. TP53 mutations are more frequently observed in high-grade invasive urothelial carcinomas. The loss or dysfunction of p53 function leads to impaired DNA damage response and cell cycle control, resulting in genomic instability and increased tumor aggressiveness (21). In urothelial carcinoma, TP53 mutations are associated with poor prognosis and resistance to chemotherapy (22). Targeting mutant p53 or restoring wild-type p53 function represents a potential therapeutic approach for urothelial carcinoma treatment (23).

In addition to FGFR3 and TP53, other genes frequently implicated in urothelial carcinoma include ERBB2 (HER2), PIK3CA (phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha), and KDM6A (lysine-specific demethylase 6A) (24, 25). ERBB2 amplifications and overexpression have been observed in a subset of urothelial carcinomas, particularly in muscle-invasive and metastatic cases (26). Targeting ERBB2 with anti-HER2 therapies, such as trastuzumab and pertuzumab, has shown promise in selected patients with ERBB2-positive urothelial carcinoma (27). PIK3CA mutations, which activate the PI3K/AKT/mTOR signaling pathway, are present in a subset of urothelial carcinomas and may predict response to targeted therapies (28). KDM6A is an X-linked histone demethylase that is frequently mutated or deleted in urothelial carcinoma, and its loss is associated with a more aggressive phenotype (29).

Qin et al. (2020) conducted a review focusing on the development of FGFR inhibitors in combination with immune checkpoint inhibitors for the treatment of urothelial carcinoma. The study discussed the rationale behind combining these two classes of drugs and highlighted the potential synergistic effects. It emphasized the potential of FGFR inhibitors as a therapeutic option to enhance the efficacy of immune checkpoint inhibitors in urothelial carcinoma (17). Baldia et al. (2016) investigated FGFR alterations in squamous differentiated bladder cancer and identified them as a potential therapeutic target in this specific subtype. The study suggested that FGFR-targeted therapies may benefit a subset of squamous differentiated bladder cancer patients, indicating the potential for personalized treatment approaches (31).

Wu et al. (2019) examined the significance of TP53 mutation in bladder cancer progression and its impact on treatment decisions. The study highlighted the role of TP53

mutations as a prognostic factor and emphasized their importance in guiding therapeutic strategies for bladder cancer patients (32). Borowczak et al. (2023) evaluated the prognostic role of p53 and its correlation with CDK9 in urothelial carcinoma. The study investigated the expression of p53 and CDK9 and their association with clinical outcomes. The findings suggested a potential prognostic value for p53 and its correlation with CDK9 expression in predicting disease progression and patient outcomes (33).

Jenkins et al. (2022) focused on HER2 overexpression and amplification in uterine carcinosarcomas with serous morphology. The study identified the presence of HER2 alterations in a subset of uterine carcinosarcomas with serous morphology, highlighting the potential for HER2-targeted treatment strategies in this specific subtype (34). Yorozu et al. (2020) assessed the HER2 status in molecular subtypes of urothelial carcinoma of the renal pelvis and ureter. The study investigated the prevalence of HER2 alterations in different molecular subtypes and their potential association with clinical characteristics, contributing to the understanding of the molecular heterogeneity of urothelial carcinoma (35).

Tharin et al. (2023) analyzed the PIK3CA and PIK3R1 tumor mutational landscape in a pan-cancer patient cohort, exploring the frequency and types of mutations and their relevance in guiding treatment decisions across various cancer types (36). Moreover, Ross et al. (2013) conducted a study focused on exploring the PIK3CA mutation spectrum in urothelial carcinoma. The researchers aimed to understand the different types of PIK3CA mutations present in this type of cancer and how these mutations contribute to signaling pathways and phenotypic outcomes (37).

Thus, somatic mutations in oncogenes and tumor suppressor genes, such as FGFR3, TP53, ERBB2, PIK3CA, and KDM6A, contribute to the pathogenesis of urothelial carcinoma by driving abnormal cell growth, survival, and invasion. These genetic alterations have important clinical implications, as they can serve as potential diagnostic and prognostic biomarkers, as well as therapeutic targets for precision medicine approaches in urothelial carcinoma treatment. Table 1 shows key genetic mutations in urothelial carcinoma.

Tumor genomics

Tumor genomics plays a crucial role in understanding the genetic landscape of urothelial carcinoma, encompassing various genetic alterations like somatic mutations, copy number alterations, and chromosomal rearrangements. The advent of next-generation sequencing (NGS) technologies has revolutionized the field by enabling comprehensive analysis of the tumor genome and transcriptome, leading to the discovery of novel driver mutations and potential therapeutic targets.

In urothelial carcinoma, the identification of actionable mutations has emerged as one of the most promising applications of tumor genomics. These actionable mutations are specific genetic alterations that can be targeted by drugs designed to inhibit or modulate the activity of the affected genes or pathways. For instance, the FGFR inhibitor erdafitinib has received approval for the treatment of advanced urothelial carcinoma with FGFR alterations (40). FGFR alterations, such as activating mutations or gene fusions, occur in a subset of urothelial carcinomas and can be identified through genomic profiling (41). Clinical trials have demonstrated the efficacy of erdafitinib in patients with FGFR-altered urothelial carcinoma, highlighting the importance of genomic profiling in guiding targeted therapy selection (42).

Another promising therapeutic approach in urothelial carcinoma is the use of poly(ADP-ribose) polymerase (PARP) inhibitors (43). PARP inhibitors exploit the concept of synthetic lethality, where cancer cells with defects in DNA repair pathways—such as those harboring mutations in DNA repair genes like BRCA1 and BRCA2—become highly dependent on PARP-mediated DNA repair mechanisms (44). Clinical trials have shown promising results for the PARP inhibitor olaparib in urothelial carcinoma patients with DNA repair gene mutations, providing a potential targeted treatment option for this subset of patients (45).

Another promising biomarker for monitoring tumor burden and treatment response in urothelial carcinoma has emerged: analyzing circulating tumor DNA (ctDNA) (46), which is composed by small fragments of DNA that are released into the bloodstream by tumor cells (47). By analy-

Table 1. Key Gene Mutations in Urothelial Carcinoma and their Clinical Implications.

Gene	Function/Pathway Involved	Frequency of Mutations in Urothelial Carcinoma	Clinical Implications	References
FGFR3	Activation of FGFR3 signaling pathway	Frequently mutated in low-grade non-invasive urothelial carcinoma	Potential therapeutic target; FGFR inhibitors under investigation	(16, 30, 31)
TP53	DNA damage response and cell cycle control	Commonly found with high frequency in high-grade invasive urothelial carcinoma	Associated with poor prognosis and resistance to chemotherapy	(32, 33)
ERBB2 (HER2)	Cell proliferation and survival	Amplification and overexpression in subset of urothelial carcinomas	Target of anti-HER2 therapies; promising in ERBB2-positive cases	(34, 35)
PIK3CA	Activation of PI3K/AKT/mTOR signaling	Mutations present in a subset of urothelial carcinomas	Predictive of response to targeted therapies	(36, 37)
KDM6A	X-linked histone demethylase	Frequent mutation or deletion in urothelial carcinoma	Associated with aggressive phenotype; potential therapeutic target	(38, 39)

zing ctDNA, real-time information on the genetic alterations present in the tumor can be obtained non-invasively (48). Several studies have demonstrated the potential of ctDNA analysis in predicting treatment response, monitoring disease progression, and detecting minimal residual disease in urothelial carcinoma patients (46). This approach holds promise for tailoring treatment strategies, assessing treatment efficacy, and detecting disease recurrence. For example, a study by Powles et al. (2021) investigated the utility of ctDNA analysis in predicting response to immune checkpoint inhibitors in metastatic urothelial carcinoma patients (49). The study found that patients with detectable ctDNA at baseline had worse outcomes compared to those with undetectable ctDNA. Moreover, changes in ctDNA levels during treatment were correlated with treatment response and survival outcomes. Similarly, another study demonstrated the potential of ctDNA analysis in detecting minimal residual disease after radical cystectomy in urothelial carcinoma patients, with ctDNA detection being associated with an increased risk of disease recurrence (46). Integration of genomic profiling, including ctDNA analysis, into routine clinical practice holds promise for improving patient outcomes and optimizing treatment strategies in urothelial carcinoma (50).

Lotan et al. compared urine markers and cytology for surveillance of patients with urothelial carcinoma (UC) using the Cxbladder Monitor test (51). This test exhibited superior sensitivity and negative predictive value compared to cytology, NMP22 ELISA, and NMP22 BladderChek tests, with consistent negative results for false negatives across all markers. In another study, Zeng et al. detected chromosomal aberrations in urothelial carcinoma using whole-genome sequencing technology and proved that the customized bioinformatics workflow had high performance in identifying chromosomal aberrations (52). Table 2 presents the findings derived from clinical trials, prospective studies, and retrospective studies utilized in the investigation of UC (urothelial carcinoma).

Loriot et al. (2019) aimed to assess the efficacy of erdafitinib, an FGFR inhibitor, in patients with locally advanced or metastatic urothelial carcinoma. The study demonstrated promising outcomes, particularly in terms of response rates and progression-free survival. Erdafitinib emerged as an effective targeted therapy for patients with FGFR-altered advanced urothelial carcinoma, highlighting its potential as a treatment option in this patient population (53). Sweis et al. (2018) conducted a clinical trial to investigate the use of olaparib, a PARP inhibitor, in urothelial bladder cancer patients with DNA damage response gene mutations. The study observed the clinical activity of olaparib in this specific subgroup of patients, suggesting its potential as a targeted therapy. The findings provided valuable insights into the personalized treatment approach for urothelial carcinoma based on the presence of DNA repair gene mutations (54).

Fenner (2021) performed a prospective study that focused on utilizing ctDNA (circulating tumor DNA) as a biomarker in urothelial cancer. The study revealed that detectable ctDNA at baseline was associated with worse outcomes, and changes in ctDNA levels correlated with treatment response and survival. This finding emphasized the potential utility of ctDNA as a valuable tool for monitoring disease progression and evaluating treatment response in urothelial carcinoma patients (55). Moreover, Das (2021) carried out another prospective study that explored the role of ctDNA detection as a predictor of disease recurrence after radical cystectomy in urothelial carcinoma patients. The study found an association between the presence of ctDNA and an increased risk of disease recurrence, suggesting its potential as a prognostic marker in this setting (56), while Faltas et al. (2017) conducted a retrospective study with the aim of identifying recurrent genetic alterations in urothelial carcinoma. The study revealed the presence of somatic mutations and copy number alterations, which were found to be associated with pathology outcomes. These findings suggested the existence of distinct molecular subtypes within urothelial carcinoma, providing valuable insights into the genetic landscape of the disease (57).

In addition, Al-Ahmadie and Iyer (2015) conducted a prospective study utilizing integrated genomic analysis to characterize subtypes of urothelial carcinoma based on their distinct molecular characteristics. The study highlighted the prognostic implications of genetic alterations and gene expression patterns in urothelial carcinoma. This comprehensive understanding of the genetic landscape and molecular heterogeneity of the disease has the potential to improve diagnosis and guide treatment decisions (58).

Liu et al. (2022) investigated the molecular mechanisms underlying urothelial carcinoma with FGFR3 mutations. The study aimed at understanding the specific genetic alterations and molecular pathways associated with FGFR3, providing valuable insights into potential targeted therapies for this particular subtype of urothelial carcinoma (59).

Al-Obaidy and Cheng (2021) explored the pathogenesis and treatment implications of fibroblast growth factor receptor (FGFR) gene alterations in urothelial carcinoma. The study shed light on the role of FGFR gene alterations in the development and progression of the disease, paving the way for potential targeted therapies aimed at this specific molecular target (60).

An interesting study by Al-Ahmadie and Iyer (2018) provided updates on the genetics and molecular subtypes of urothelial carcinoma, including select variants, through a comprehensive review. The review emphasized the importance of understanding the genetic landscape and molecular heterogeneity of urothelial carcinoma for improved diagnosis and personalized treatment strategies (61).

Pharmacogenomics

Pharmacogenomics focuses on how drug response and toxicity are impacted by genetic variations: the pharmacokinetics and pharmacodynamics of drugs can be influenced by genetic variations in drug-metabolizing enzymes, drug transporters, and drug targets, thus leading to variable drug efficacy and toxicity, which plays a crucial role in personalized medicine approaches for urothelial carcinoma.

The association between genetic variations and treatment outcomes has been extensively studied in urothelial carcinoma patients receiving chemotherapy and immunotherapy. In a Phase II study, cisplatin-gemcitabine regimen with bevacizumab showed promising overall radiographic response rates and improved median overall survival com-

Table 2 displays evidence from clinical trials and prospective and retrospective studies for UC.

Gene Mutation	Type of study	Target therapy	References
FGFR alterations	Clinical Trial	Erdafitinib, an FGFR inhibitor, showed efficacy in FGFR-altered advanced urothelial carcinoma.	(53)
DNA repair gene mutations	Clinical Trial	Olaparib, a PARP inhibitor, demonstrated promising results in urothelial carcinoma with DNA repair gene mutations.	(54)
ctDNA	Prospective Study	Detectable ctDNA at baseline correlated with worse outcomes, and changes in ctDNA levels correlated with treatment response and survival outcomes.	(55)
ctDNA	Prospective Study	ctDNA detection was associated with an increased risk of disease recurrence after radical cystectomy.	(56)
Somatic mutations, copy number alterations	Retrospective Study	Identified recurrent genetic alterations in urothelial carcinoma and their association with patient outcomes.	(57)
Genetic alterations, gene expression	Prospective Study	Integrated genomic analysis revealed subtypes of urothelial carcinoma with distinct molecular characteristics and prognostic implications.	(58)
FGFR3, TP53	Retrospective Study	Investigated the relationship between FGFR3 and TP53 mutations and their association with patient outcomes and response to therapy.	(59, 60)
Genomic alterations, molecular subtypes	Integrative Molecular Analysis	Identified molecular subtypes of urothelial carcinoma with distinct genomic alterations and clinical characteristics.	(61)
Somatic mutations, copy number alterations	Integrative Molecular Analysis	Identified recurrent genetic alterations in urothelial carcinoma and their association with patient outcomes.	(62, 63)
DNA repair gene alterations	Prospective Study	Detected DNA repair gene alterations in urothelial carcinoma and their association with prognosis and response to chemotherapy.	(64, 65)

pared to chemotherapy alone (66). Ongoing Phase III trials are further evaluating these regimens in advanced urothelial carcinoma patients (50). The prognostic significance of the VEGF axis, particularly VEGFA, has been supported in urothelial cancer, and understanding angiogenesis can aid in theragnostics and patient stratification (67).

In the realm of immunotherapy, immune checkpoint inhibitors have been approved for bladder cancer treatment, but response rates remain modest (65). PD-L1 expression in tumor samples has shown promise as a potential biomarker for immunotherapy response, although its use as a selection criterion is not widely implemented (68). Mutational load, reflecting the number of mutations in the tumor genome, has also emerged as a predictive biomarker for immunotherapy response, as higher mutational load has been correlated with increased likelihood of response to anti-PD-L1 antibodies (69). Other genetic variations—such as those in the CYP2D6 gene and drug transporter genes like ABC transporters—have been explored as potential predictive markers for chemotherapy outcomes (70). Genome-wide association studies (GWAS) have provided insights into the genetic basis of chemotherapy response in urothelial carcinoma, identifying genetic loci associated with treatment outcomes (71).

Biological therapies

Immunotherapy presents a promising approach for treating UC, using drugs that stimulate the immune system to target cancer cells. For example, the treatment of UC has been revolutionized by immune checkpoint inhibitors (ICIs), a type of immunotherapy that has gained approval (72). ICIs function by blocking proteins that hinder the immune system's ability to attack cancer cells, thereby enhancing immune recognition and response against cancer cells. Multiple clinical trials have demonstrated the efficacy of ICIs in UC treatment. In a study, patients with metastatic UC who received the ICI pembrolizumab exhibited higher response rates and longer progression-free survival compared to those who received chemotherapy (73). Another study highlighted that the ICI atezolizumab improved overall survival in patients with locally advanced or metastatic UC (72). ICIs generally exhibit better tolerability with fewer side effects when compared to chemotherapy.

Monoclonal antibodies (mAbs) also demonstrate promise in UC treatment. These targeted drugs are designed to bind to specific proteins on the surface of cancer cells, aiding in their destruction or impeding their growth and spread. Atezolizumab, an approved mAb for UC treatment, targets PD-L1, a protein expressed on some cancer cells. By blocking PD-L1, atezolizumab activates the immune system to attack cancer cells (74). Other mAbs-targeting proteins (such as Nectin-4, which is frequently overexpressed in UC cases), like enfortumab vedotin, have shown favorable results in clinical trials and have gained approval for locally advanced or metastatic UC treatment (75).

Emerging in UC treatment are biomarker-driven strategies that leverage biomarkers like genetic mutations or protein expression levels to identify patients likely to benefit from specific treatments. FGFR3 mutations have been studied as a biomarker in UC, present in approximately 20% of cases, and associated with a more favorable response to FGFR inhibitors (75). Several FGFR inhibitors are under development for UC treatment, with promising

results observed in clinical trials involving patients with FGFR3 mutations. Another biomarker of interest in UC is PD-L1 expression, which may indicate a higher likelihood of response to ICIs (72). However, PD-L1 expression alone does not guarantee response, prompting the exploration of other biomarkers to identify patients who will benefit most from ICIs.

Proteomic biomarkers

Proteomics is a rapidly evolving field that aims to identify and quantify the entire set of proteins expressed by a cell, tissue, or organism. Proteomic biomarkers have the potential to provide valuable information about the molecular mechanisms underlying UC development and progression, as well as to predict treatment response and clinical outcomes. In recent years, several studies have focused on identifying proteomic biomarkers in UC using various techniques, such as mass spectrometry, immunohistochemistry, and bioinformatics.

One of the most promising proteomic biomarkers in UC is fibroblast growth factor receptor 3 (FGFR3), a transmembrane receptor that regulates cell proliferation and differentiation. FGFR3 mutations are present in up to 80% of low-grade non-invasive UC cases and are associated with a favorable prognosis (76). Moreover, FGFR3 expression levels have been shown to correlate with tumor grade, stage, and recurrence in UC patients. Therefore, FGFR3 has been proposed as a potential diagnostic and prognostic biomarker in UC.

Another proteomic biomarker that has attracted attention in UC is aquaporin-1 (AQP1), a water channel protein that plays a role in cell migration and invasion (77). AQP1 overexpression has been observed in high-grade invasive UC and is associated with poor survival outcomes. Furthermore, AQP1 has been shown to enhance the sensitivity of UC cells to cisplatin, a commonly used chemotherapy drug. Therefore, AQP1 may serve as a predictive biomarker for chemotherapy response in UC patients.

Researchers designed another study to identify plasma protein biomarkers for early diagnosis of bladder carcinoma (78). They employed 2D-DIGE and mass spectrometry techniques, which led to the identification of fifteen differentially expressed proteins. Among them, haptoglobin exhibited high sensitivity and specificity in distinguishing between low-grade bladder cancer patients and controls. These findings indicate the potential of haptoglobin and other identified proteins as biomarkers for early detection of bladder cancer, emphasizing the need for further validation and investigation. Moreover, another study used plasma samples from bladder cancer patients and compared these to normal samples using 2-dimensional SDS-PAGE, image gel analysis, and MALDI-TOF mass spectrometry, resulting in the identification of three groups of proteins with altered abundance (11). The first group included modified forms of plasma transferrin, fibrinogen gamma, and complement C3b, absent in normal plasma, while the second group comprised proteins such as haptoglobin, alpha-2-macroglobulin, vitamin D-binding protein, and pigment epithelium-derived factor, found in higher quantities in cancerous samples. The third group consisted of three molecular forms of immunoglobulin M (IgM), significantly lower in relative abundance in cancerous plasma samples. Table 3 describes the role of protein biomarkers, metabolites, and microbes in urothelial cancers.

Metabolomic and microbiomic prognostic indicators

Metabolomic profiling of urine samples has demonstrated its potential in predicting the prognosis of urothelial carcinoma. Several studies have investigated the metabolomic profile of urine samples from urothelial carcinoma patients, aiming to identify metabolites that correlate with disease progression and patient outcomes. For example, in a study by Issaq et al (79), urine samples from 48 healthy individuals and 41 patients with transitional cell carcinoma (bladder cancer) were analyzed using a high-performance liquid chromatography-mass spectrometry approach.

The statistical analysis, using positive ionization mass spectrometry, accurately predicted the status of all 48 healthy urine samples and all 41 bladder cancer urine samples, demonstrating a sensitivity and specificity of 100% for bladder cancer detection. Moreover, their analysis also supported these results, correctly identifying 46 out of 48 healthy urine samples and 40 out of 41 bladder cancer urine samples. A study by Pasikanti et al. (2017) analyzed urine samples from urothelial carcinoma patients and identified alterations in metabolites associated with amino acid metabolism, lipid metabolism, and energy metabolism. Furthermore, they found that specific metabolites, including creatinine, taurine, and citrate, exhibited significant associations with patient prognosis, suggesting their potential as prognostic biomarkers. Pasikanti et al. conducted a study to investigate urinary metabotyping of bladder cancer using two-dimensional gas chromatography time-of-flight mass spectrometry (GC×GC-TOFMS) (80). They analyzed urine samples from bladder cancer patients and non-cancer controls. The OPLS-DA model demonstrated high specificity (100%) and sensitivity (71%) in detecting bladder cancer, outperforming cytology. The study also identified metabolites and perturbed metabolic pathways associated with bladder cancer, including alterations in the tryptophan-quinolinic metabolic axis. In another work, gas chromatography/time-of-flight mass spectrometry was used for urinary metabolic profiling of 24 BC patients and 51 non-BC controls (81). Multivariate analysis, including principal component analysis and OPLS-DA, demonstrated a clear differentiation between BC patients and non-BC subjects based on global urinary metabolic profiles. Urinary metabonomics achieved 100% sensitivity in detecting BC, outperforming urinary cytology which achieved only 33% sensitivity.

In addition to metabolomics, microbiomic analysis has shown promise as a prognostic indicator in urothelial carcinoma. Several studies have explored the association between microbial communities and clinical outcomes in urothelial carcinoma patients. Numerous studies have shown that microbial populations can exert an influence on urological conditions, indicating the potential involvement of microbes in the continuum of health and disease states (85). The precise nature and role of these microbes are still

Table 3. Role of Protein Biomarkers, Metabolomics, and Microbiomics in Urothelial Cancer: Key Findings from Selected Studies

Study	Focus	Key Findings	References
Lemaska-Perek et al. (2019)	Protein Biomarkers	Identified potential plasma biomarkers of bladder cancer through proteomic analysis, which could aid in diagnosing and monitoring the disease.	(11)
Blanca et al. (2016)	Protein Biomarkers	Identified FGFR3 and Cyclin D3 as urine biomarkers for bladder cancer recurrence.	(76)
Morrissey et al. (2016)	Protein Biomarkers	Examined urine aquaporin-1 and perilipin-2 concentrations as biomarkers for renal cell carcinoma screening.	(77)
Nedjad et al. (2015)	Protein Biomarkers	Discovered a circulating proteomic signature for detecting biomarkers in bladder cancer patients.	(78)
Amara et al. (2019)	Metabolomics	Discussed the use of metabolomics in bladder cancer research, highlighting its potential for identifying metabolic alterations and developing diagnostic and therapeutic strategies.	(14)
Issaq et al. (2008)	Metabolomics	Detected bladder cancer in human urine by metabolomic profiling.	(79)
Pasikanti et al. (2013)	Metabolomics	Urinary metabotyping of bladder cancer using metabolomic analysis.	(80)
Pasikanti et al. (2010)	Metabolomics	Developed a noninvasive urinary metabonomic diagnostic model for bladder cancer.	(81)
Alfano et al. (2016)	Microbiomics	Explored the interplay between the extracellular matrix and the microbiome in urothelial bladder cancer, emphasizing the complex interactions and their implications in cancer development and progression.	(16)
Buevi Popovi et al. (2018)	Microbiomics	Investigated the urinary microbiome associated with bladder cancer, providing insights into the potential role of microbiota in the disease.	(82)
Wu et al. (2018)	Microbiomics	Identified specific microbial species, such as Acinetobacter baumannii, in bladder cancer patients, suggesting their potential involvement in the disease.	(83)
McConnell et al. (2013)	Microbiomics	Discussed Gram-positive anaerobic cocci as opportunistic pathogens associated with urothelial cancer.	(84)

under investigation, but their impact on bladder cancer carcinogenesis has been evident in the long-standing observation of the association between squamous cell carcinoma of the bladder and urogenital schistosomiasis. S. haematobium, in particular, has consistently been reported to be associated with this type of bladder cancer, potentially contributing to pathogenesis through mechanisms such as epithelial damage, chronic inflammation, and oxidative stress (86).

Despite the significance of microbial involvement in bladder cancer, only a limited number of studies have reported detailed analyses of the urinary microenvironment in urothelial bladder cancer. In one study, Xu et al. compared the urine microbiota of healthy individuals and that of bladder cancer patients, and observed an enrichment of Streptococcus in the urine of patients with urothelial carcinoma (87). Streptococcus abundance was nearly absent in most healthy individuals, and, in cases where Streptococcus abundance was low in cancer samples, Pseudomonas or Anaerococcus were the most abundant genera. However, the study had limitations due to its small sample size. Another similar study compared bacterial communities in urine samples from healthy individuals and from cancer patients, revealing Firmicutes as the most abundant phylum in both groups, followed by Actinobacteria, Bacteroidetes, and Proteobacteria. Operational taxonomic units (OTUs) belonging to the genus Fusobacterium were found to be more abundant in the bladder cancer group (82). Confirming these findings, an independent analysis of 42 bladder cancer tissues detected Fusobacterium nucleatum sequences using protein chain reaction in 11 samples. Additionally, the genera Veillonella, Streptococcus, and Corynebacterium were found to be more abundant in healthy urine samples.

In recent investigations, patients with bladder cancer demonstrated an increase in bacterial richness, defined by the number of unique OTUs in a sample. This greater bacterial richness was also observed in urine from patients with non-muscle invasive bladder cancer (NMIBC) who had a high risk of recurrence or progression, based on the European Organization for Research and Treatment of Cancer (EORTC) scoring system (83). Therefore, higher bacterial richness may serve as a potential indicator of the high risk of recurrence and progression in NMIBC. Notably, Acinetobacter and Anaerococcus were found to be more abundant in bladder cancer patients compared to the non-cancer group (83). Acinetobacter baumannii, known for its virulence factors, can invade epithelial cells, degrade phospholipids, and form biofilms, enabling evasion from the host immune response (84). Anaerococcus, a member of the Gram-positive anaerobic cocci, has been reported to induce inflammation and remodeling of the extracellular matrix (ECM) (88). The research work proposes that the interplay between the ECM, microbiome, and inflammation plays a crucial role in the onset, progression, and relapse of bladder cancer (16).

Conclusions

The advent of omics sciences has ushered in a new era in our comprehension of urothelial carcinoma, offering

unprecedented opportunities for advancements in diagnosis, prognosis, and treatment. The comprehensive exploration of tumor genomics, pharmacogenomics, biological therapies, proteomic biomarkers, and metabolomic and microbiomic prognostic indicators has illuminated the path towards refined strategies for managing urothelial carcinoma. Nonetheless, the complexity inherent in the heterogeneity of urothelial carcinoma necessitates the establishment of standardized assays and rigorous biomarker validation protocols. Moving forward, it is crucial for future research endeavors to concentrate on forging personalized approaches to urothelial carcinoma by integrating multifaceted omics data with clinical parameters, while simultaneously identifying novel therapeutic targets and devising innovative strategies to overcome resistance mechanisms.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review

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Omics sciences and precision medicine in thyroid cancer

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Abstract

Background. Thyroid cancer, a heterogeneous disease originating from the thyroid gland, stands as the predominant endocrine malignancy worldwide. Despite advances in diagnosis and treatment, some patients still experience recurrence and mortality, which highlights the need for more personalized approaches to treatment. Omics sciences, encompassing genomics, transcriptomics, proteomics, and metabolomics, offer a high-throughput and impartial methodology for investigating the molecular signatures of thyroid cancer.

Methods. In the course of this review, we have adopted a focused research strategy, meticulously selecting the most pertinent and emblematic articles related to the topic. Our methodology included a systematic examination of the scientific literature to guarantee a thorough and precise synthesis of the existing sources.

Results. These techniques enable the identification of molecular markers that can aid in diagnosis, prognosis, and treatment selection. As an illustration, through genomics studies, numerous genetic alterations commonly discovered in thyroid cancer have been identified, such as mutations in the BRAF and RAS genes. Through transcriptomics studies, distinctively expressed genes in thyroid cancer have been uncovered, playing roles in diverse biological processes, including cell proliferation, invasion, and metastasis. These genes can serve as potential targets for novel therapies. Proteomics studies have unveiled differentially expressed proteins intricately involved in thyroid cancer pathogenesis, presenting promising biomarkers for early detection and disease progression monitoring. Metabolomics studies have identified alterations in metabolic pathways linked to thyroid cancer, offering promising avenues for potential therapeutic targets.

Conclusions. Precision medicine in thyroid cancer involves the integration of omics sciences with clinical data to develop personalized treatment plans for patients. Employing targeted therapies guided by molecular markers has exhibited promising outcomes in enhancing the prognosis of thyroid cancer patients. Notably, those with advanced thyroid cancer carrying BRAF mutations have displayed substantial responses to specific targeted therapies, such as vemurafenib and dabrafenib. ***Clin Ter 2023; 174 Suppl. 2 (6):11-20 doi: 10.7417/CT.2023.2467***

Key words: *thyroid cancer, biomarker, genomics, metabolomics, precision medicine, diagnosis, treatment*

Introduction

The thyroid gland, the initial endocrine gland to develop in humans, is a well-vascularized structure situated in the base of neck. With a soft and reddish appearance, it assumes the shape of an H. Utilizing iodine, the thyroid gland synthesizes essential hormones (T3, T4) responsible for regulating vital bodily functions, including heart rate, blood pressure, body temperature, and basal metabolic rate (1).

Thyroid cancer stands as the most frequently diagnosed form of endocrine cancer, surpassing all other types of endocrine cancer combined in its impact on mortality (2): in fact, it accounts for the majority (95%) of all endocrine-related cancers (3). Symptoms of thyroid cancer encompass a neck lump or swelling, difficulties in swallowing or breathing, and changes in voice hoarseness (4). Despite its relative rarity, constituting approximately 2% of all cancers (3), thyroid cancer can affect individuals of all ages, genders, and ethnicities.

As thyroid cancer is not very frequent, it is difficult to pinpoint its exact etiology (5). Nevertheless, numerous risk factors have been identified, including radiation exposure, a family history of thyroid cancer, and specific inherited genetic abnormalities (6). Thyroid cancer, second only to

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ovarian cancer, ranks as the most lethal form of cancer originating in the endocrine system. Despite comprising only 1% of all cancer-related deaths, it still remains a substantial concern (7). The objective of this review is to present an encompassing overview of the current state of knowledge in the field of omics sciences and precision medicine in thyroid cancer, incorporating the most recent research and advancements.

Various types of thyroid carcinomas and their histological characteristics

Thyroid cancer is conventionally categorized into two main groups, determined by clinical and morphological characteristics: differentiated cancer—which encompasses papillary (PTC), follicular (FTC), and medullary carcinomas (MTC)—and undifferentiated thyroid cancer, referred to as anaplastic thyroid carcinoma (ATC) (6). Over the last thirty years, there has been a rapid surge in the worldwide incidence of thyroid cancer (8). Roughly two-thirds of all cases, in both males and females, fall under the category of papillary thyroid cancer. Follicular thyroid cancer constitutes 10-20% of cases, while medullary thyroid cancer accounts for 5-10% of the total cases. Anaplastic thyroid cancer is the least common, comprising less than 5% of all cases (9). Papillary (PTC), follicular (FTC), and anaplastic (ATC) thyroid cancers arise from the thyroid follicular epithelial cells, whereas medullary thyroid cancer (MTC) originates from the parafollicular C-cells (10).

Papillary thyroid cancer stands as the most common type, with a generally favorable prognosis. This form of cancer tends to metastasize more frequently to the lymph nodes in the neck than to the lungs. On the other hand, follicular thyroid cancer and poorly differentiated thyroid cancers are categorized as high-risk cancers due to their propensity for spreading through the bloodstream to distant sites, particularly the lungs and bones. The staging system for these types of cancers varies based on age, with patients aged 45 and older experiencing less favorable prognoses (11). Medullary thyroid cancer originates in the parafollicular cells, the neuroendocrine cells of the thyroid gland. Typically, it presents as a solitary lump in the thyroid gland, predominantly affecting individuals aged between 40 and 60 (12). In some cases, the first symptom of medullary thyroid cancer is swelling of the lymph nodes in the neck, as the disease commonly spreads to the cervical lymph nodes. Approximately 70% of patients with detectable medullary thyroid cancer exhibit signs of metastasis to the cervical lymph nodes during surgery (13). Certain patients may manifest symptoms of a thyroid nodule, alongside flushing and diarrhea, suggesting a possible cancer spread. Around 25% of medullary thyroid cancer cases are associated with patients inheriting multiple endocrine neoplasia syndrome (12).

Anaplastic thyroid cancer is an infrequent and rapidly growing type of thyroid cancer, often presenting as a sizable, firm lump in the neck (14). Common symptoms include hoarseness, difficulty swallowing, and shortness of breath. If a patient exhibits a suspicious mass, an immediate biopsy is crucial for proper diagnosis. Additional tests may indicate that the cancer has metastasized to other regions of the body, with the lungs, bones, and brain being the most prevalent sites. Anaplastic thyroid cancer may either arise from differentiated thyroid cancer or manifest independently. When a patient with a history of thyroid cancer exhibits these symptoms, anaplastic transformation should be considered as a possibility. Given the unfavorable prognosis associated with this cancer, referring patients to a specialized treatment center is advisable. Fortunately, clinical trials are actively investigating novel treatments for anaplastic thyroid cancer, holding the potential to offer hope for improved survival rates (15).

Insights into the Genetic Basis of Thyroid Carcinoma

Emerging findings from DNA sequencing studies have revealed that the MAPK signaling pathway harbors genetic mutations, predominantly contributing to the occurrence of thyroid cancers. Functioning as a vital conduit for transmitting growth signals from the cell membrane to the nucleus, this pathway plays a pivotal role in regulating cell growth and division. The identified mutations within this pathway culminate in the unbridled proliferation of thyroid cells, ultimately fostering the development of cancer (16).

Gene mutation

A specific point mutation (T1799A) in exon 15 of the BRAF gene leads to the expression of a mutant protein known as BRAF-V600E, resulting in the constitutive activation of a serine/threonine kinase (17, 18). This mutation represents one of the most prevalent genetic alterations observed in thyroid cancer, occurring in approximately 45% of sporadic papillary thyroid carcinoma (PTC) cases and up to 80-100% of tall cell variant PTC cases. The presence of the BRAFV600E mutation correlates with more aggressive pathological features and higher recurrence rates, serving as a predictor for poor clinical outcomes (19). Additionally, this mutation is recognized for causing a loss of radioiodine avidity, rendering it less responsive to radioiodine treatment.

Point mutations in RAS genes are a common occurrence not only in thyroid cancer but also in various other types of solid cancers. Among the three isoforms of RAS (HRAS, KRAS, and NRAS), NRAS is the most frequently mutated in thyroid tumors. While RAS mutations are relatively rare in typical papillary thyroid carcinoma (PTC) cases (0-20%), almost half of follicular thyroid carcinoma (FTC) and follicular variant PTC cases carry RAS mutations. Moreover, approximately 20% of follicular adenoma cases show RAS mutation, suggesting its early involvement in thyroid tumorigenesis. The RAS mutation leads to a reduction in GTPase activity, resulting in a constitutively active state. Additionally, RAS mutation activates the PI3K-AKT pathway, playing a significant role in the development of thyroid cancer.

The tumor suppressor gene PTEN plays a crucial role in regulating the PI3K-AKT signaling pathway, acting as a significant negative regulator in opposition to PI3K. Mutation or deletion of PTEN has been linked to the tumorigenesis of follicular thyroid cells, evident in Cowden's syndrome—an autosomal inherited disease caused by germ line mutations

of the PTEN gene. Cowden's syndrome exhibits a strong correlation with an elevated risk of thyroid, breast, and endometrial cancers, as well as benign hamartomas. PTEN alterations are frequently detected in around 40% of follicular thyroid carcinoma cases. Furthermore, promoter hypermethylation has been identified as a mechanism for PTEN silencing, observed in both follicular thyroid carcinoma and anaplastic thyroid carcinoma (20). Thyroid cancers can involve mutations in various genes, including CTNNB1, TP53, IDH1, and NDUFA13 (GRIM19). CTNNB1 is a gene that plays a role in the WNT-β-catenin pathway and is frequently found to be mutated in anaplastic thyroid cancer (ATC) (21). The TP53 gene is responsible for producing the tumor suppressor protein p53 and is associated with several types of solid tumors. In cases of anaplastic thyroid cancer (ATC), TP53 mutations are commonly identified, with a frequency ranging from 70% to 80% (22, 23). CTNNB1 and TP53 mutations are predominantly observed in poorly differentiated thyroid carcinoma or anaplastic thyroid carcinoma, suggesting that these genetic alterations may occur later in the cancer progression derived from follicular cells. In contrast, Hürthle cell thyroid cancer typically lacks the typical genetic mutations seen in other types of thyroid cancer, such as BRAFV600E, RAS, or RET/PTC (24). Instead, around 15% of Hürthle cell thyroid cancer cases exhibit mutations in NDUFA13 (also known as GRIM19) (25). Thyroid carcinoma-related gene mutations and alteration are reported in Table 1.

Genetic predisposition stands as one of the factors contributing to thyroid cancer. In cases where the cancer is hereditary, there is a higher likelihood of recurrence and increased aggressiveness, often manifesting at a younger age compared to sporadic thyroid cancer (38, 39). Thyroid cancer can be categorized into two main types: medullary thyroid cancer, originating from parafollicular cells, and non-medullary thyroid cancer (NMTC), originating from follicular cells. NMTC is the most prevalent form, accounting for 95-97% of all cases. Within NMTC, there is a specific subtype called hereditary NMTC, which has a genetic basis and represents approximately 5-15% of NMTC cases and 3-9% of all thyroid cancers (40, 41). Genes that make individuals more susceptible to NMTC can also cause other diseases besides thyroid cancer. This parallels observations in other cancer syndromes, such as familial adenomatous polyposis (FAP) resulting from APC gene mutations (42), Cowden syndrome arising from PTEN gene mutations (43, 44), and Carney complex associated with PRKAR1A gene mutations (44). Regular monitoring and timely surgical intervention can help high-risk cancer patients. Genetic testing enables the identification of individuals at risk, facilitating targeted preventive treatment for the appropriate population and avoiding unnecessary treatment for others. Despite the widespread adoption of genetic testing in clinical practice, the genes that elevate susceptibility to NMTC are frequently overlooked (45), and testing for these genes is not yet a routine part of clinical settings (46).

Among individuals diagnosed with papillary thyroid cancer, at most 10% have a family member (either a first or second-degree relative) who has also experienced PTC. It is essential to highlight that PTC is not a prevalent form of cancer, ranking as the ninth most common cancer in the United States, with an estimated 44,670 new cases reported in 2010 (47). While a 10% rate of familial occurrence may seem relatively high, it's important to acknowledge that in most cases, this is based on only one or two additional cases besides the original person. Pedigrees exhibiting Mendelian-type inheritance, where a trait is clearly inherited in a predictable pattern, are exceptionally uncommon, and pedigrees with more than five affected individuals are particularly rare. Even in families where inheritance follows a Mendelian pattern, it is common for the trait to skip generations or remain unexpressed (nonpenetrance). Considering these observations, it is probable that multiple genes are involved in predisposition to PTC, exhibiting low penetrance, while environmental factors also contribute to the equation. It cannot be completely ruled out that there are Mendelian genes with high penetrance that predispose individuals to PTC; however, based on available evidence, it appears that if such genes do exist, they are not common. Such rare genes may be categorized as "common disease, rare allele," as elucidated by Bodmer and Bonilla (48). Currently, association studies are limited to identifying genes that have common variations (known as single nucleotide polymorphisms or SNPs) with a frequency of over 1% in

Table 1. Thyroid Carcinoma-Related Gene Mutations and Alteration

Genes Involved	OMIM	Correlated Pathology	Inheritance	References
RET	164761	Papillary Thyroid Carcinoma	Autosomal Dominant	(26)
BRAF	164757	Papillary Thyroid Carcinoma	Sporadic	(27)
NTRK1	191315	Papillary Thyroid Carcinoma	Sporadic	(28)
TP53	191170	Anaplastic Thyroid Carcinoma	Autosomal Dominant	(29)
PTEN	601728	Follicular Thyroid Carcinoma	Autosomal Dominant	(30)
NKX2-1	600635	Poorly Differentiated Thyroid Carcinoma	Autosomal Dominant	(31)
RAS	190070	Differentiated Thyroid Carcinoma	Sporadic or Autosomal Dominant	(32)
TERT	187270	Differentiated Thyroid Carcinoma	Sporadic	(33)
TP53	191170	Follicular thyroid carcinoma, Papillary thyroid carcinoma	Autosomal dominant	(34)
MET	164860	Papillary thyroid carcinoma	Autosomal dominant	(35)
p16	600160	Papillary thyroid carcinoma	Autosomal dominant	(36)

the population. However, certain genes with rare variations that are only present in a few families cannot be detected through these studies. In the pursuit of these genes, researchers employ a technique called linkage analysis, involving the examination of the DNA of extensive families. This approach has proven fruitful in the case of PTC, leading to the identification of several potential gene locations (49).

Somatic mutations

The development of papillary thyroid cancer (PTC) is influenced by diverse genetic factors. Notably, mutations within the BRAF and RAS genes are involved, activating the MAPK signaling pathway and present in 40% and 15% of PTC cases, respectively (RAS mutations are exclusively found in the follicular variant). Moreover, rearrangement of the RET/PTC gene is detected in 18% of PTC cases. These mutations are mutually exclusive and correspond to distinct characteristics and behaviors of the tumors (50). Follicular thyroid carcinoma (FTC) diverges from papillary thyroid carcinoma (PTC) in terms of genetic mutations. FTC is frequently associated with mutations in either the RAS or PTEN genes, or rearrangements in the PAX8/PPAR genes. These genetic abnormalities are prevalent in a significant percentage of FTC tumors, ranging from 50% to 80% (51). RAS mutations are not limited to malignant follicular carcinoma alone, as they can also be found in benign follicular adenomas. Nevertheless, the mechanisms underlying the progression of follicular adenomas to follicular carcinoma remain poorly understood (51).

Epigenetic modifications

Initially, the term "epigenetic" was coined to describe the way genes interact with the environment, resulting in observable traits. Over time, its scope has expanded to encompass the study of mechanisms controlling changes in gene expression that can be transmitted through somatic cells or even germ cells. These changes cannot be attributed to alterations in DNA sequence (52). The process of epigenesis is extensively studied, particularly concerning the addition of a methyl group to the 5-carbon position of cytosine. This modification is carried out by a group of enzymes known as methyltransferases, which target cytosines within a CpG dinucleotide sequence. The methyl group is provided by S-adenosyl-L-methionine. In vertebrates, cytosine methylation represents the sole known methylation reaction (53). The process of epigenesis is most extensively studied in relation to the addition of a methyl group to the 5-carbon position of cytosine. This process is carried out by a group of enzymes called methyltransferases, which target cytosines that are part of a CpG dinucleotide sequence. The methyl group is provided by S-adenosyl-L-methionine. In vertebrates, methylation of cytosine is the only known methylation reaction that occurs (54). During the process of development, specific patterns of gene expression that are unique to each tissue are established. These patterns are closely linked to specific patterns of methylation or demethylation of CpG islands that are located near the gene promoters (55). Research findings suggest a correlation between the expression of the thyroglobulin gene in thyroid tissue and the unmethylated state of its promoter, indicating the absence of chemical modifications to the DNA that could suppress gene expression. In simpler terms, the regulation of this gene's expression in thyroid tissue hinges on the unmethylated status of its promoter region, which is essential for enabling appropriate expression (56).

Research has demonstrated that GABP, a transcription factor widely present in the body, is involved in regulating the transcription of the TSHR gene in a manner dependent on methylation. Specifically, certain CpG sites within the TSHR promoter region must be methylated for GABP to effectively regulate gene expression. In rat FRT thyroid cells, which do not express the TSHR gene, the lack of expression appears to be influenced by both the methylation of these specific CpG sites and the methylation sensitivity of GABP. However, in rat thyroid cells (FRTL-5) expressing the TSHR gene, these CpG sites are entirely demethylated, enabling GABP to bind and effectively regulate the expression of the TSHR gene (57).

Tumor genomics (tumor-typical somatic mutations and circulating tumor DNA)

Most of the genetic alterations underlying different types of thyroid cancer have been elucidated. These changes fall into two categories: somatic mutations or somatic copy number alterations (SCNAs), and they display an inverse relationship, with tumors often exhibiting either a high number of somatic mutations or a high number of SCNAs, but rarely both. Somatic mutations in thyroid tumors mainly affect crucial elements of three primary oncogenic pathways: (i) RTK-RAS-RAF, (ii) PI3K-AKT-mTOR, and (iii) components of the cell cycle or the DNA repair machinery. In thyroid tumors, there is a relatively high proportion of somatic mutations, indicating numerous genetic changes occurring in the DNA of non-reproductive cells throughout a person's lifetime. On the other hand, the percentage of somatic copy number alterations (SCNAs) is low, comprising only about 30% of genetic alterations in these tumors (58). While copy number alterations (SCNAs) are not prevalent in thyroid cancer, they play a substantial role in approximately one-third of papillary thyroid carcinomas (PTCs) lacking gene fusions or driver mutations. This suggests that SCNAs alone can be oncogenic. Likewise, in poorly differentiated and anaplastic thyroid carcinomas (PDTC and ATC, respectively), SCNAs are more frequent in patients without driver mutations. SCNAs can activate oncogenes and suppress tumor suppressor genes, as they often encompass large DNA regions containing multiple genes.

As an illustration, the loss of the 22q region is present in around 10% of PTCs, with the majority of these cases being part of the follicular variant, which is enriched in RAS mutations. SCNAs can influence different gene regions in cancer cells, and one such region that may be affected is the chromosomal region 1q, which is gained in approximately 15% of all papillary thyroid carcinomas (PTCs). This gain has been correlated with the presence of BRAF mutations and is linked to a heightened risk of cancer recurrence and a

poorer prognosis in poorly differentiated thyroid carcinoma (PDTC). However, despite the association, no specific genes linked to this particular region have been identified. On the other hand, in anaplastic thyroid carcinoma (ATC), the loss of 8p and 17p regions, along with gains in the 20q region, are commonly observed. These SCNAs have been correlated with a more unfavorable prognosis for ATC patients (59).

Cell-free DNA (cfDNA) consists of small fragments of DNA released by cells, present in the bloodstream. Under normal circumstances, cfDNA originates from the natural breakdown of blood cells (60). However, in cancer patients, a portion of cfDNA is produced due to the death of cancer cells, known as circulating tumor DNA (ctDNA) (61). ctDNA possesses unique properties, such as its size and stability, and contains genetic and epigenetic information that reflects the tumor's characteristics (62). This renders ctDNA a valuable source of information for cancer detection and monitoring, as well as for devising personalized treatments (63).

Assessing the amount, quality, genetic alterations, and epigenetic modifications of cf-DNA can be beneficial in determining the diagnosis of disseminated tumor cells (64). It seems that individuals who have been diagnosed with differential thyroid cancer (DTC) have higher levels of cf-DNA quantity and quality as compared to those who have not been affected by the disease (65). Conversely, the amount of mitochondrial cell-free DNA (mcf-DNA) is reduced in the same group of patients (66).

Extensive research has focused on the presence of the BRAFV600E mutation in various human cancers, with melanoma and thyroid cancer being the most common types where it is observed. However, recent studies have revealed its detection in lung cancer as well (67). Investigations indicate that the BRAFV600E mutation may be associated with advanced thyroid cancer, elevating the likelihood of nodal and distant metastasis (68). The majority of studies on thyroid cancer cfDNA have concentrated on using PCR to detect specific point mutations in circulating DNA. However, two studies have explored the methylation of specific genes in cfDNA, deemed a more reliable indicator due to its relative stability compared to point mutations. Moreover, there exists a wide range of mutations associated with each cancer type, each occurring with relatively low incidence (69). Studies have demonstrated that the average detection rate of the BRAFV600E mutation in circulating cfDNA among DTC patients is 10%. This rate rises to 19.3% when patients with non-BRAFV600E tumors are excluded. However, the relatively low detection rate may be attributed to the inclusion of tumors at different stages, with early-stage tumors potentially not shedding sufficient tumor DNA into the circulation to be detectable using current techniques. Encouragingly, promising evidence for the potential use of cfDNA as a diagnostic tool in thyroid cancer comes from Pupilli et al., who discovered a substantially higher proportion of BRAFV600E cfDNA in PTC patients compared to those with benign nodules. They also found a higher proportion in individuals with suspicious cytology in comparison to those with benign cytology (11).

Research has revealed that the quantity of cfDNA (cell-free DNA) in the bloodstream is associated with the stage of various cancers. Moreover, the rate of cfDNA release into the bloodstream corresponds to the size of the primary tumor (70). A study indicates that the levels of circulating cfDNA with BRAFV600E mutation seem to be correlated with the occurrence of nodal and distant metastasis in PTC (71). A meta-analysis revealed a correlation between the BRAFV600E mutation and advanced clinical stage in various medical conditions (72). The identification of BRAFV600E in ctDNA holds promise as a non-invasive marker for identifying aggressive thyroid cancer. Detecting BRAFV600E in a patient's blood could indicate the presence of aggressive disease. Additionally, cfDNA can serve as a target for developing new methods to detect cancer recurrence after treatment.

At present, surveillance for thyroid cancer recurrence involves neck ultrasound scans and measuring thyroglobulin levels. However, this can be challenging due to the presence of antibodies in a substantial number of patients, which may interfere with accurate thyroglobulin measurements (73). Scientists researching various types of cancer have reported that a decrease in the amount of total cfDNA in a patient's body during treatment can be indicative of their response to the administered therapy (74). This statement indicates that the research conducted on thyroid cancer measured the levels before and after the treatment, and the results remained consistent in both instances (75).

Pharmacogenomics for thyroid cancer

Tumor pharmacogenomics is the investigation of how a person's genetic makeup affects their reaction to various cancer medications. These genetic alterations may also impact the efficacy of radiation therapy, and it is crucial to comprehend the role of pharmacogenomics in determining which patients are suitable for treatment when developing new radiopharmaceutical therapies for cancer patients (76).

For more than 40 years, the main treatment for well-differentiated thyroid cancer has involved surgery followed by ^{131}I therapy (when deemed appropriate). However, in some patients, ^{131}I therapy may not work, and some metastatic lesions may not take up the radioactive iodine. This could be due to a reduction in the NaI symporter in tumor cells. In such cases, inducing NaI expression and restoring responsiveness to ^{131}I therapy through redifferentiation therapy may be effective. Research has demonstrated that drugs targeting tyrosine kinases in tumors with NRAS and BRAFV600E mutations can facilitate the administration of successful ^{131}I treatment in affected patients (77).

An alternative treatment approach for thyroid cancers is external beam radiation (EBM) therapy, utilizing high-energy beams or particles to eliminate cancerous cells or hinder their growth (78). Typically, radiation therapy is not recommended for patients with differentiated thyroid cancers (DTCs) who respond well to radioactive iodine (RAI) therapy. However, in the case of patients with medullary thyroid cancer (MTC) or anaplastic thyroid cancer (ATC), EBM and chemotherapy are commonly employed in their treatment. Chemotherapy involves administering anti-cancer drugs through injection, infusion, or orally, which then travel through the bloodstream to target and eliminate cancer cells. Nonetheless, chemotherapy is generally not effective for most types of thyroid tumors and is typically used in

conjunction with EBM therapy for ATC or for advanced thyroid cancer patients who have not responded to other treatments.

In recent years, significant progress has been made in the development of new medications tailored to specifically target the molecules responsible for tumor formation, known as targeted therapy (79). Distinguishing themselves from traditional chemotherapy drugs, targeted therapy drugs focus on specific molecular pathways in cancer cells, rather than simply attacking fast-growing cells. Within the realm of thyroid cancer therapies, various small molecules have been identified as potential targets, falling into three main categories: oncogenic kinases, signaling kinases, and vasculature/angiogenesis processes. Furthermore, there are other molecules that target epigenetic mechanisms (such as Fosbretabulin, Romidepsin, Celecoxib, Vorinostat, Valproic acid, Azacytidine, and Decitabine) as well as nuclear receptors.

Vandetanib (Caprelsa) is a medication prescribed to manage symptomatic and aggressive medullary thyroid cancer (MTC). This targeted therapy is available in the form of a 300mg biconvex, oval-shaped tablet. Cabozantinib (Cometriq), on the other hand, is a small molecule targeted therapy used to treat MTC and as a second-line treatment for renal cell carcinoma. It predominantly inhibits tyrosine kinases c-Met and VEGFR2, exerting greater effects on these targets compared to AXL and RET (80, 81). Cabozantinib has demonstrated its effectiveness in inhibiting cancer growth in MTC patients, with a duration of approximately seven months longer than a placebo. In the treatment of radioiodine-refractory differentiated thyroid cancer, two targeted therapy drugs, Lenvatinib (Lenvima®) and sorafenib (Nexavar®), function as kinase inhibitors and have been utilized (82, 83). Dabrafenib and Trametinib are combined medications utilized to treat advanced melanoma and anaplastic thyroid carcinoma (ATC). They function by inhibiting the formation of new blood vessels in cancer cells and targeting essential proteins crucial for cancer cell growth (84, 85). This combination therapy proves to be a viable treatment option for ATC patients with a specific type of positive BRAF gene mutation and for those who have not undergone complete tumor removal through surgery (86).

Metabolomic and microbiomic prognostic indicators

Metabolomics is a burgeoning field of research in thyroid cancer, still in its early stages compared to genomics and proteomics, resulting in fewer studies. The most commonly utilized techniques include mass spectrometry and NMR spectrometry, with HRMAS NMR demonstrating potential in identifying various thyroid lesions (87). Most studies have primarily focused on analyzing tissue specimens, with limited research on less invasive biological fluids (such as serum, plasma, or urine) as diagnostic biomarkers for thyroid cancer (88). Despite this, all studies have successfully distinguished between cancerous and normal tissues, as well as benign and malignant lesions.

In a study by Shang et al., they aimed to profile the metabolites of 25 papillary carcinoma tissues and compared them to 25 healthy controls, employing both targeted and untargeted approaches. For untargeted analysis of 15 cancerous and normal samples, they utilized gas chromatography-time of flight mass spectrometry (GC-TOF-MS), while for targeted analysis of 10 paired samples, they utilized ultra-high-performance liquid chromatography-triple-quadrupole mass spectrometry (UHPLC-QqQ-MS) and GC-TOF-MS. In their untargeted analysis, they successfully identified 45 significant metabolites, such as oleic acid, sorbitol, galactinol, arachidic acid, glutaric acid, melibiose, glucose, linolenic acid, uridine, and melatonin. To validate these findings, the subsequent targeted analysis confirmed the significance of certain metabolites, with sorbitol, glucose, galactinol, melibiose, and melatonin standing out as the most notable (89). In this study, a non-targeted approach involving gas chromatography and mass spectrometry was employed to compare thyroid tissues from 16 patients with papillary thyroid carcinoma (PTC) and normal thyroid tissues. To confirm the metabolic changes observed, an RT-qPCR experiment was conducted to assess enzyme genes. The results revealed several significant alterations in metabolites. Notably, there was a decrease in carbohydrates such as glucose, fructose, galactose, mannose, and rhamnose, along with reduced malonic acid and increased inosine concentration related to nucleotide metabolism. Moreover, lipid metabolism showed an increase in cholesterol and arachidonic acid. The study also highlighted notable elevations in mRNA levels of G6PD, PGK1, LDHA, PHGDH, and PTGS2 (90).

In a particular study, researchers conducted a comparison between benign and malignant thyroid tissues, which revealed distinct metabolic differences. Malignant tissues exhibited elevated levels of phosphocholine, glycerophosphocholine, phosphoethanolamine, lactate, and various amino acids, while showing decreased levels of citrate, scyllo- and myo-inositol, inosine, and uridine. Moreover, when comparing malignant and benign nodules, the study identified increased levels of uracil, hypoxanthine, xanthine, and amino acids, with decreased levels of choline. The observed alterations mainly involved changes in energy metabolism, such as glycolysis, lipid metabolism, and the TCA cycle, as well as in protein turnover, nucleotide biosynthesis, and phosphatidylcholine biosynthesis (91).

The serum biomarkers that have been identified demonstrate a remarkable ability to accurately diagnose cancer patients, effectively distinguishing them from healthy individuals, and accurately differentiating between those with malignant and benign thyroid lesions. These findings highlight the potential of metabolomics to significantly advance our comprehension of the molecular mechanisms underlying thyroid cancer (TC), rendering it a highly promising research avenue for studying this disease. Furthermore, through metabolomics studies, we can gain deeper insights into cancer-related processes and uncover novel biomarkers, which have the potential to drive improvements in TC diagnosis and classification.

Microbiomics correlation with Thyroid Cancer

Microbes present in tumor tissues of papillary thyroid carcinoma (PTC), but not in adjacent normal tissues, are believed to have a significant impact on immune cell expression

and the regulation of immune and cancer pathways, thereby potentially restraining cancer growth. Notably, these microbes seem to be more abundant in tall cell and male patient groups, showing a correlation with heightened expression of mutations and tumor suppressor methylation (93).

Substantial evidence suggests that the composition of microorganisms in the gut (the gut microbiome) could increase susceptibility to certain types of cancer, alter the functioning of the human immune system, and influence the tumor microenvironment's response to treatment (94). Manipulating the microbiome in a targeted way has shown potential in enhancing the effectiveness of PD-1 blockade, indicating that the microbiome may play a role in supporting therapeutic approaches for tumors (95).

The gut microbiome has emerged as a crucial regulator of thyroid function as a host factor. Research has demonstrated that germ-free rats, raised in a sterile environment without gut bacteria, exhibit smaller thyroid glands compared to conventionally raised rats, highlighting the substantial influence of gut microbes on thyroid health (96). Furthermore, imbalances in the intestines have been linked to both low thyroid function and autoimmune thyroid conditions, further underlining the association between gut microbiota and thyroid-related disorders (97). The gut microbiome has been linked to both thyroid cancer (TC) and the development of thyroid nodules, indicating its potential role in thyroid health (98). Specifically, individuals with high-grade thyroid nodules have shown a gut microbiome with increased amino acid breakdown and decreased butyrate production, implying a connection between gut bacteria and thyroid problems through host-microbe metabolite interactions (99). Moreover, gut bacteria contribute to the conversion of thyroxine (T4) to triiodothyronine (T3) in the intestine and can modulate the immune response of T helper 1 (Th1) and Th2 cells (100). Microorganisms also influence thyroid hormone levels by regulating iodine cycling, highlighting their impact on thyroid function within the body and the intricate interactions between the host and gut microbiome (97).

Proteomic Studies

Proteomics has gained popularity in the search for novel protein biomarkers for cancer diagnosis and prognosis (101). In PTC research, scientists frequently employ techniques such as two-dimensional electrophoresis, differential in-gel electrophoresis, and liquid chromatography to separate intricate protein mixtures. The identified proteins are then analyzed using mass spectrometry and database searches. Proteomics methods are often complemented by other techniques, including northern and western blotting, as well as immunohistochemical staining. For more precise quantification of protein levels, enzyme-linked immunosorbent assay (ELISA) is commonly utilized. These integrated approaches offer valuable insights into the study of PTC and its potential biomarkers.

Scientists use a comparative approach to identify differentially expressed proteins in tissue and serum samples of PTC patients compared to healthy individuals or those with benign thyroid goiter, using proteomics methods (102). Additionally, proteomics has been applied to analyze proteins produced by PTC cell lines, as well as cyst fluid and urine samples. Among the early proteomics studies on PTC, researchers reported an upregulation of prohibitin and ATP synthase D chain in tissue samples of patients compared to controls (102). These findings highlight the potential of proteomics in unraveling the protein signatures associated with PTC and its various sample sources, facilitating the discovery of new biomarkers and therapeutic targets.

In recent advances, researchers have successfully obtained protein profiles of thyroid cancer in humans by employing specific tumor cell lines. The process involves subjecting glycoproteins to precise denaturation procedures, followed by digestion and solid-phase extraction for extraction purposes. Identification of these proteins is achieved through electrospray ionization tandem mass spectrometry (ESI-MS/MS) analysis. Preliminary results have unveiled variations in the protein composition of thyroid cancer, contingent upon the particular type of cancer under investigation (103). These protein profiling techniques offer valuable insights into the molecular intricacies of thyroid cancer and hold promise for developing targeted therapies and personalized treatment approaches.

It was discovered that thyroid cell lines do not exhibit the same traits as in vivo tumors. Although these cell lines were derived from various tumor types, they have developed phenotypes and gene expression profiles that are similar to undifferentiated tumors due to in vitro evolution. This has led to the absence of expression of most thyrocyte-specific genes, non-responsiveness to thyrotropin, and a high number of chromosomal abnormalities. While thyroid cell lines retain certain properties of the cells they originated from, such as genetics, epigenetics, and gene expression, they differ significantly from in vivo tumors. Therefore, when interpreting thyroid cell line studies, it's important to consider these findings (104).

Conclusion

In conclusion, omics sciences have brought about a paradigm shift in the diagnosis, treatment, and control of thyroid cancer. The advent of diverse omics technologies has significantly enhanced our comprehension of the molecular underpinnings of thyroid cancer, fueling the advancement of precision medicine. By leveraging omics sciences, we have successfully pinpointed specific gene mutations and somatic alterations, enabling the development of targeted interventions like pharmacogenomics and biological therapies. Furthermore, the identification of protein biomarkers and of metabolomic and microbiomic prognostic indicators has improved the accuracy of thyroid cancer diagnosis and prognosis.

In summary, omics sciences have provided new routes for research and improved the clinical management of thyroid cancer, leading to better outcomes for patients.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review *Clin Ter 2023; 174 Suppl. 2 (6):21-28 doi: 10.7417/CT.2023.2468*

Omics sciences and precision medicine in testicular cancer

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Abstract

Background. Cancer, a potentially fatal condition, is one of the leading causes of death worldwide. Among males aged 20 to 35, the most common cancer in healthy individuals is testicular cancer, accounting for 1% to 2% of all cancers in men.

Methods. Throughout this review, we have employed a targeted research approach, carefully handpicking the most representative and relevant articles on the subject. Our methodology involved a systematic review of the scientific literature to ensure a comprehensive and accurate overview of the available sources.

Results. The onset and spread of testicular cancer are significantly influenced by genetic changes, including mutations in oncogenes, tumor suppressor genes, and DNA repair genes. As a result of identifying these specific genetic mutations in cancers, targeted medications have been developed to disrupt the signaling pathways affected by these genetic changes. To improve the diagnosis and treatment of this disease, it is crucial to understand its natural and clinical histories.

Conclusions. In order to comprehend cancer better and to discover new biomarkers and therapeutic targets, oncologists are increasingly employing omics methods, such as genomics, transcriptomics, proteomics, and metabolomics. Targeted medications that focus on specific genetic pathways and mutations hold promise for advancing the diagnosis and management of this disease. ***Clin Ter 2023; 174 Suppl. 2 (6):21-28 doi: 10.7417/CT.2023.2468***

Key words: *Cancer, Testicular cancer, genomics, metabolomics, diagnosis, biomarker*

Introduction

The most common cancer among healthy males aged 20 to 35 is testicular cancer, accounting for 1% to 2% of all cancers in men. The most well-established risk factor for testicular cancer is cryptorchidism, which refers to the condition of undescended male testis. Studies have reported a risk ratio (RR) range between 3.5 and 17.1 for testicular cancer in individuals with cryptorchidism. Other significant risk factors for testicular cancer include a prior diagnosis in the opposite testis, with reported risk (1). Vasectomy, scrotal injury, and inguinal hernia have not been established as significant risk factors for testicular cancer. However, a recent systematic study has found a minor but statistically meaningful association between an increased risk of testicular cancer and subfertility. The annual incidence of testicular cancer is approximately 4 per 100,000 people, and it is rare among individuals without underlying risk factors, most of which are highly treatable (2).

Testicular cancer is the second most prevalent new cancer diagnosis among Canadians aged 15 to 29, with 14% of all cancer diagnoses (3). The age range of 15 to 29 poses specific challenges for young cancer patients seeking treatment, as it falls between the fields of pediatric oncology and medical oncology focused on older adults. Germ-cell neoplasms are the primary type of testicular malignancies. Among these, approximately half are seminomas, while the remaining half are non-seminomas. Differentiating between these histological types is crucial, as non-seminoma tumors have a higher likelihood of spreading. Understanding the causes

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of these tumors and determining the most effective treatment approaches relies on this histological distinction. Testicular cancer is showing an increasing incidence, although its causes are not yet fully comprehended. There are a few recognized risk factors for testicular cancer, such as premature birth and cryptorchidism (undescended testicle). However, despite the discontinuation of DES (diethylstilbestrol) prescriptions since the early 1970s, the prevalence of testicular cancer has continued to rise. It is widely believed that germ cell neoplasia in situ (GCNIS) precedes the development of almost all testicular cancers (4).

The causal relationship between maternal smoking and testicular cancer has not been definitively proven. However, the risk of testicular cancer is known to be increased by heavy or prolonged cigarette smoking. Furthermore, there is a correlation between smoking status and the aggressiveness of testicular tumors (5). Excessive or prolonged cannabis use, similarly to tobacco use, has been associated with an increased risk of testicular cancer, particularly of the nonseminoma subtype. This risk is further increased if cannabis use begins before the age of 18. Additionally, certain dietary changes, such as consuming a high amount of fat and dairy products, have been suggested as potential risk factors for testicular cancer. It is also noted that increased prosperity often leads to an increase in sedentary behavior, which has been linked to the disease (6).

Testicular cancer is a complex malignancy, characterized by its heterogeneity and distinct clinical subtypes. The advent of omics technologies has revolutionized cancer research and provided unprecedented opportunities to unravel the molecular intricacies of various cancers, including testicular cancer (7). Omics approaches, such as genomics, transcriptomics, proteomics, and metabolomics, have been instrumental in elucidating the underlying mechanisms, identifying biomarkers, and advancing personalized medicine in the field of testicular cancer (8).

Genomics, as a key omics discipline, has enabled the comprehensive characterization of the genetic landscape of testicular cancer. Through genome-wide studies, numerous genomic alterations have been identified, including chromosomal aberrations, copy number variations, and gene mutations. These genomic aberrations have provided valuable insights into the pathogenesis of testicular cancer, highlighting critical oncogenic drivers and tumor suppressor genes involved in tumorigenesis and disease progression (9). Transcriptomics, the study of gene expression patterns, has significantly contributed to our understanding of testicular cancer biology. By employing techniques such as microarray analysis and RNA sequencing, researchers have identified specific gene expression signatures associated with different subtypes of testicular cancer. These gene expression profiles not only facilitated accurate classification of testicular tumors, but also provided insights into the molecular processes driving tumor development, metastasis, and treatment response (10).

Proteomics, which is the study of proteins and their interactions, has played a vital role in uncovering the protein networks and signaling pathways involved in testicular cancer. Proteomic analyses have identified proteins that are differentially expressed in testicular cancer tissues compared to normal testicular tissues. Furthermore, proteomics has been instrumental in identifying potential protein biomarkers for diagnostic, prognostic, and therapeutic purposes. These biomarkers hold promise for improving early detection, predicting treatment outcomes, and guiding personalized treatment strategies (11).

Metabolomics, the study of small molecule metabolites, has emerged as a powerful tool for understanding the metabolic alterations associated with testicular cancer. Metabolomic profiling of testicular cancer samples has revealed perturbations in various metabolic pathways, providing insights into the energy metabolism, nutrient utilization, and biosynthetic processes in testicular tumors. These findings may have implications for the development of targeted therapies aimed at disrupting specific metabolic pathways or exploiting metabolic vulnerabilities in testicular cancer cells (12).

Introduction to tumor biology

Cancer, a potentially fatal condition, remains one of the leading causes of death globally. The rate of advancement in our understanding of cancer is remarkable; however, the more we delve into its details, the more complex it appears. It is crucial to understand its underlying mechanisms, as they operate in a dynamic and interconnected manner at both the molecular and cellular levels. This understanding is essential in our ongoing fight against this disease (13). Big omics data, also known as cancer systems biology, can be produced by investigating global DNA, RNA, and protein expression, which in turn lead to a systems approach to cancer biology research, addressing the complexity of cancer by combining experimental and computational techniques in the synthesis and testing of cancer biological theories (14).

Recent years have seen a substantial improvement in our knowledge about tumor biology, natural history, clinical history, and genetics, which has sparked the development of novel diagnostic and therapeutic strategies. Testicular cancer propensity can be identified through genetic testing, which also offers useful information for customizing treatment choices (15). Additionally, the tumor's genetics, which includes gene fusions, rearrangements, and somatic mutations, can direct targeted therapy. Proteomic, metabolomic, and microbiomic profiling, together with other newly discovered biomarkers, may offer further details on the pathophysiology and individualized treatment of testicular cancer (16).

Genetics of testicular cancer (germline mutations that predispose to cancer)

Processes of DNA damage (both exogenous and endogenous) as well as DNA repair leave their imprint on the genomes of cancerous cells. By examining the somatic mutation landscape within these cells, we can identify the mutational forces responsible for oncogenesis. Certain mutational signatures have well-established etiologies, and understanding these signatures can provide insights into the underlying causes of testicular cancer (17).

Due to deficiencies in DNA repair systems, exposure to exogenous mutagens, or errors in DNA replication, so-

matic mutations can manifest in various forms, known as mutational signatures. These mutational signatures can be extracted from a tumor's diversity and examined to elucidate potential factors contributing to its etiology (18). Comparing variations in mutational signatures based on the age of onset can be particularly valuable, because cancer risk and latency periods of exposure differ across age groups. This is crucial, given that testicular cancer is a rare form of cancer that is challenging to assess rapidly in a traditional epidemiological study setting. Analyzing the mutational landscape can also provide insights into treatment approaches and help understanding the factors contributing to the increasing incidence of testicular cancer among young individuals. For instance, studies have shown that the mutational burden influences the response to immunotherapy (19).

In a previous analysis conducted by (Wheeler et al 2013), molecular alterations in testicular cancer tumors identified three genes involved in somatic mutations in testicular cancers, which are KIT, KRAS, and NRAS. The study also observed that the most common type of base changes observed were cytosine to thymine. Interestingly, the authors noted that the mutational signature known as the "COSMIC signature," which arises from the accumulation of 5-methylcytosine deamination events, coincided with the most prevalent mutational signature in testicular cancer (20).

The use of genomics in the treatment of cancer has rapidly increased in recent years. Through genomic research, it is possible to identify frequent genetic changes that occur in cancer, such as chromosomal rearrangements, fusion genes, and somatic mutations (21). Chromosomal rearrangements, including translocations or deletions, are common genetic events in cancer that can cause altered expression or function of genes involved in cell proliferation, differentiation, and apoptosis, resulting in the onset and progression of the disease. Fusion genes, which are formed as a result of chromosomal rearrangements, influence the growth and survival of cells by activating or inactivating specific pathways. Somatic mutations, which are genetic changes that take place in non-germline cells, can also impact the genes responsible for carrying out cellular functions and aid in the growth of cancer (22).

The use of liquid biopsies, which examine circulating tumor cells or circulating tumor DNA (ctDNA) in the blood, is another aspect of genomics in tumor care that has drawn a lot of interest. A promising biomarker for cancer diagnosis, prognosis, and therapy response monitoring is ctDNA analysis. It can offer details on the chromosomal rearrangements and somatic mutations that are present in the tumor, which can help determine the best targeted therapy (23).

Tumor genomics (tumor-typical somatic mutations and circulating tumor DNA)

Testicular cancer is not specifically connected to any one gene, although it is highly heritable and can be passed from parent to child. Additionally, if a first-degree relative has testicular cancer, the average age of diagnosis is two to three years lower than the general population (24). The onset and spread of testicular cancer are significantly influenced by genetic changes. In testicular cancer, mutations in oncogenes, tumor suppressor genes, and DNA repair genes have been frequently identified. These genetic alterations play a significant role in the development and progression of testicular cancer, leading to the development of targeted therapeutics for testicular cancer as a result of the discovery of these genetic abnormalities. Over the past few decades, there have been major developments in the study of genetics, which have resulted in the identification of numerous genetic variants that affect tumor initiation and progression (25). Genetic testing has become an integral component of tumor management, due to its ability to identify individuals who are predisposed to certain types of cancers. By analyzing an individual's genetic makeup, genetic testing can provide valuable information about their susceptibility to specific cancer types. This information can be instrumental in developing personalized treatment plans, implementing preventive measures, and offering genetic counseling to at-risk individuals and their families (26).

Numerous studies have proven that specific genetic variations are associated with an increased chance of developing certain types of cancer. Colorectal, endometrial, and other cancers are more likely to develop in people who have Lynch syndrome, which is brought on by mutations in DNA mismatch repair genes. Genetic testing can detect somatic alterations in the tumor tissue in addition to germline mutations. Somatic mutations are not inherited and take place in non-germline cells (27). These mutations, which are specific to the tumor tissue, can reveal details about the biology, behavior, and potential therapeutic response of the tumor. Targeted medicines that can selectively target somatic mutations have been developed as a result of the detection of somatic mutations (28).

Table 1. Testicular cancer Genetic Mutations and their phenotypes.

Gene	Genetic Mutation(s)	Inheritance	Phenotype	OMIM
TP53	Various Mutations	AD	Li-Fraumeni syndrome	191170
KIT	C-KIT Mutations	AD	Familial testicular germ cell tumors	164920
BRCA2	Various Mutations	AD	Hereditary breast and ovarian cancer syndrome	600185
RAD51C	RAD51C Mutation	AR	Fanconi anemia	602774
CHEK2	CHEK2 Mutations	AD	Li-Fraumeni-like syndrome	604373

Pharmacogenomics of the specific testicular tumor

The study of genetic differences that affect drug response and toxicity is known as pharmacogenomics. Drug efficacy and safety may be increased by identifying genetic variants that impact drug transport, pharmacodynamics, and metabolism. Pharmacogenomics can also help with drug selection and dosage, which results in more individualized therapy options (29). The analysis of genetic variants that affect drug toxicity and response is becoming more crucial in the treatment of tumors. Drug efficacy and safety may be increased by identifying genetic variants that impact drug transport, pharmacodynamics, and metabolism. Pharmacogenomics can also help with drug selection and dosage, which will benefit cancer patients' prognoses. The management of tumors now places a high priority on genetics and pharmacogenomics (30). Technology advancements have made it possible to pinpoint specific genetic changes in cancers and create tailored medicines that can enhance therapeutic outcomes. Pharmacogenomics and the use of liquid biopsies have both shown promise in the diagnosis, prognosis, and therapy of cancer (31). It is possible to enhance individualized cancer treatment and eventually improve patient outcomes by incorporating genetics and pharmacogenomics into clinical practice (32).

Enzymes that regulate the metabolism, uptake, and reaction to numerous clinically used medications, such as bleomicin, etoposide, and platins, have been the main focus of pharmacogenomic research on testicular cancer, in light of the fact that Cytochrome P450 is responsible for the majority of antineoplastic medication metabolism and that treatment efficiency is frequently impacted by variant alleles in these enzymes. It is important to adequately address the study of these phase I and phase II enzymes in order to support the development of therapeutically more effective medicines (33).

Biological therapies (immunotherapy, monoclonal antibody therapy)

When determining the appropriate course of treatment for testicular tumors, both the stage and subtype of the tumor are to be considered. While orchiectomy followed by monitoring is a suitable and frequent therapeutic option for these patients, metastatic testicular tumors are normally treated with chemotherapy alone or in combination with chemotherapy, surgery, and, in a few uncommon situations, radiation therapy (34). Approximately 15-25% of patients with metastatic disease relapse after beginning the treatment. However, salvage treatment can cure 50% of this patient group. The last category of patients includes those with cisplatin-resistant diseases or those who relapsed after receiving second-line treatment. The disease has a terrible prognosis for these patients, who are typically treated with cutting-edge chemotherapy regimens. Despite numerous therapeutic strategies incorporating targeted and biological therapies have been attempted in cisplatin-refractory testicular malignancies, conventional chemotherapy with low efficacy is still employed for these patients (33).

Immunotherapy

The primary reasons why the mammalian testes are considered immunologically privileged locations are their unique immunological milieu, which shields germ cells from autoimmune attack, and a lack in the testicular immune system's ability to respond to antigens (35). Additionally, it appears that the mechanisms governing the immune privilege of the testis also regulate spermatogenesis and steroidogenesis (36).

The phenomenon of spontaneous testicular tumor regression without therapy is thought to be related to the immune environment of the host and changed tumor vascularization. Characterizing immune cells and cytokine profiles within the tissue has allowed researchers to determine the precise immune response to the presence of in situ germ cell neoplasia (GCNIS) and overt germ cell tumors (GCT) (37). When compared to healthy testicles or inflammatory lesions associated with hypospermatogenesis, Klein et al. found that testicular germ cell tumors had a markedly distinct pattern of immune cell distribution. Patients received either pembrolizumab or nivolumab therapy (38); however, soon after receiving a single dose of medication, four individuals passed away from tumor growth. One of the three remaining patients experienced a partial radiographic response, but it's crucial to remember that this patient also received concurrent etoposide therapy (39).

As of now, immune checkpoint inhibition has not shown proven effectiveness in the treatment of refractory testicular cancer outside of clinical trials. There may be a group of patients who could benefit from immune checkpoint inhibition, according to case reports, but there are no reliable indicators available at this time for clinical decision-making in everyday practice (40). High PD-L1 expression in choriocarcinoma may be a meaningful biomarker, according to data from GTS; however, there is currently insufficient information to support such a choice in TGCTs. Alternative predictors, such as TILs and/or the tumor mutation burden, may exist; however, more study is required to assess their usefulness. To better understand the detection of prognostic markers predicting the reaction to immune-based therapy in patients with intractable testicular cancer, more research is needed (41).

Monoclonal antibody therapy

Monoclonal antibodies, which were first developed almost three decades ago, are revolutionizing the way physicians treat COVID-19 and other diseases as well as cancer. These medicines imitate the immune system's built-in defense against infection (42). Millions of y-shaped proteins known as antibodies, or antibody receptors, are produced by the immune system. Each antibody is traveling throughout the body in search of a specific target that is located on the exterior of an alien cell known as an antigen. An antibody that locates its target bonds with the antigen to aid the immune system in eliminating the sick cell (41).

Monoclonal antibodies are used to treat a variety of cancer types. They are given to patients via an infusion and

can be used either alone or in combination with other cancer treatments (43). Each monoclonal antibody may work in one or more ways depending on the antigen it is aiming to bind. Certain monoclonal antibodies target cancer cells selectively and kill them as a result. Due to their focus on specific cell receptors, these monoclonal antibodies are referred to as tailored therapies. For instance, HER2-positive breast cancer and stomach cancer are both treated with trastuzumab (Herceptin). More monoclonal antibodies improve the immune response to cancer cells (44).

Proteomic, lipidomic and metabolomic biomarkers

Testicular cancer biomarkers are essential for diagnosis, prognosis, and monitoring. Despite substantial improvements in cancer diagnosis and treatment over the past few decades, the early identification of cancer by diagnostic, prognostic, and predictive biomarkers remain one of the most promising research domains for locating early-stage cancer and adjusting therapy. The accuracy of biomarkers for illness diagnosis, treatment efficacy prediction, and tumor stage classification could all be increased. However, the individualized treatment of cancer patients is complicated by the dearth of trustworthy biomarkers (45). The variety of the oncogenic event is correlated with the heterogeneity of the distinct cancers. Additionally, diverse properties of the same histology, may lead to therapeutic failure. Because cancer is heterogeneous, the absence of a particular biomarker with 100% accuracy in diagnosis can be explained. To better define neoplasms at the molecular level, metabolomic, proteomic, and lipidomic methods are being used (46). Proteomic, lipidomic, and metabolomic biomarkers that have been studied in relation to testicular cancer include the following:

Proteomic Biomarkers: The biomarker alpha-fetoprotein (AFP) is well-known for detecting testicular cancer. Testicular cancer subtypes including non-seminomatous germ cell tumors (NSGCT) are linked to elevated levels of AFP in blood serum or testicular tumor tissue (47). Like AFP, high hCG levels have been linked to NSGCT and can be found in blood serum or urine. Increased levels of the enzyme lactate dehydrogenase (LDH) have been found in patients with testicular cancer and are linked to advanced disease stages and a bad prognosis. Several miRNAs, including "miR-371a-3p and miR-375," have demonstrated potential as biomarkers for the early identification and follow-up of testicular cancer. They can be found in biological fluids like blood serum (48).

Lipidomic Biomarkers: Lipids serve a variety of significant functions in cellular activities, such as survival, proliferation, and death because they are engaged in "chemical energy storage, cellular signaling, cell membranes, and cell-cell interactions in tissues." These cellular processes, in particular transformation, progression, and metastasis, have a close connection to carcinogenesis pathways (49). Bioactive lipids are crucial for many biological processes, and many neoplastic illnesses result in changes to their composition. Lipid changes in cancer cells have been studied by numerous earlier research teams to better understand the illness and identify potential biomarkers (50). T cells have the ability to control prostaglandin E2 PGE2 actions, which can help tumor cells evade detection. Following the use of genomics and proteomics, lipidomics was first applied in 2003 as a metabolomic technique to investigate the "qualitative and quantitative profile of the lipid components from serum, plasma, tissue, cells, and organisms (51). Testicular cancer tissues have been shown to contain altered sphingomyelin species. As lipidomic indicators for the illness, specific SM level abnormalities may exist. Testicular cancer has been linked to changes in phosphotidylcholine (PC) metabolism (52). Changes in PC composition and levels may shed light on the disease's pathophysiology. Lipidomics is an emerging technique for tumor characterization that can be used to recognize and classify neoplastic cells or tissues as well as to differentiate between a neoplasmic and normal environment. This approach can also be used to identify novel tumor biomarkers and assess the reactivity of anticancer therapies. The application of lipidomic techniques to cancer research may open up new avenues for understanding cancer diagnosis, prognosis, and the forecasting of personalized treatments (53). Table 2 lists the metabolites and biomarkers associated with testicular cancer that have drawn the most interest. Different testicular cancer subtypes and individuals may exhibit these signs to varying degrees.

Metabolomic Biomarkers: Metabolomics is a more recent addition to the omics toolkit, which is being employed more frequently in therapeutic settings. A sensitive molecular readout that is frequently connected to disease and its states, notably in cancer, is provided by small molecule assessments in tissues, blood, or urine (54). The altered metabolism of cancer, which has recently become more recognized in connection with cancer, is a significant aspect of the disease. The use of metabolomics in human breast tumors is discussed in this review, with a focus on its application in clinical diagnosis. In contrast to the clinical diagnostic techniques for breast cancer that are available now, metabolomics has the ability to detect cancer, predict outcomes of therapy (55). In the tissues of testicular cancer, higher concentrations of choline metabolites such phosphocholine and glycerophosphocholine have been found. These metabolites play a role in the reorganization of cell membranes and could be used as metabolomic indicators. Testicular cancer has been associated with elevated lactate levels, which point to altered energy metabolism (the Warburg effect) in cancer cells. Testicular cancer tissues have been found to have lower citrate levels. Citrate regulates energy metabolism, therefore changes to it may be a reflection of cancer-related metabolic abnormalities (56). Although these biomarkers have demonstrated potential in research studies, more validation and standardization are required before they can be implemented into common clinical practice for the diagnosis and treatment of testicular cancer. Additionally, compared to individual biomarkers, biomarker panels or combinations may offer enhanced sensitivity and specificity (57).

Microbiomic prognostic indicators

Microorganisms are thought to play a role in the pathogenesis of about 20% of all malignancies. The most

Table 2. Table for Metabolites and biomarkers of testicular cancer

Metabolite/Biomarker	Type	Source	Association with Testicular Cancer
Alpha-fetoprotein (AFP)	Protein	Blood serum, tumor tissue	Elevated levels associated with non-seminomatous germ cell tumors (NSGCT)
Human chorionic gonadotropin (hCG)	Protein	Blood serum, urine	Elevated levels associated with NSGCT
Lactate dehydrogenase (LDH)	Protein	Blood serum	Increased levels associated with advanced disease stages and poor prognosis
MicroRNAs (miRNAs)	RNA	Blood serum	bodily fluids Specific miRNAs like miR-371a-3p and miR-375 show potential as biomarkers for detection and monitoring
Sphingomyelin (SM)	Lipid	Testicular cancer tissue	Altered levels observed in testicular cancer tissues
Phosphatidylcholine (PC)	Lipid	Testicular cancer tissue	Changes in PC metabolism associated with testicular cancer
Choline and derivatives	Metabolite	Testicular cancer tissue	Increased levels of choline metabolites
Lactate	Metabolite	Testicular cancer tissue	Elevated levels indicate altered energy metabolism
Citrate	Metabolite	Testicular cancer tissue	Decreased levels associated with testicular cancer

prevalent kind of cancer in young men is testicular tumors, which develop from the germ cell neoplasia in situ (GCNIS) progenitor cell (58). In relation to TC, the microbiota of seminal plasma and testicular tissue has not been extensively studied. The population of microorganisms, such as bacteria, viruses, fungus, and other microbes, that live in different bodily regions, such as the gastrointestinal system, skin, and reproductive organs, is referred to as the microbiome (59). Despite the paucity of research on the microbiome's function in testicular cancers, mounting evidence points to the microbiome's potential role in the initiation and progression of cancer. However, little research has been done on particular microbiomic prognostic markers for testicular malignancies (60). According to reports, the physiological activity of the liver, gut, brain, immune cells, and several endocrine glands is influenced by the gut microbiota, which is the second biggest genome of the host. The testis and the gut microbes interact extensively. Spermine can be produced by the body's endogenous polyamine metabolism and is also absorbed from food and the gut bacteria. The activities of spermine include antioxidation, ion channel modulation, lipid synthesis inhibition, and preservation of the reproduction system's normal physiology (61).

The microbial populations in the urine and reproductive systems are referred to as the genitourinary microbiome. There is little study explicitly focusing on testicular tumors, despite studies exploring the genitourinary microbiome in relation to other urological diseases, such as bladder cancer and prostate cancer. It may be possible to find potential connections or prognostic clues by researching the genitourinary microbiome in relation to testicular cancers (61).

Cancer development and treatment response have both been linked to immune system and microbiota interactions. Immune function and how well the body respond to anticancer therapies can be affected by dysbiosis, or an imbalance in the microbiome composition. Examining the connections between the immune system and the microbiome in testicular cancer may reveal information about the disease's prognosis and possible treatments (62). It's critical to remember that the study of microbiomics and its connection to testicular tumors is still in its infancy, and further investigation is required to identify precise microbiomic prognostic indicators for testicular cancer. It may be possible to identify potential biomarkers and therapeutic targets with further research into the makeup, variety, and functional significance of the microbiome in testicular cancers (63). Intestinal bacteria were removed by antibiotics and replaced by gut microbial transplantation in order to study the effects of spermine and gut microbiota on testicular dysfunction. Following antibiotic therapy, there were fewer total bacteria and sperm in the cecum lumen, which were restored by gut microbial rebuilding (64).

Conclusion

Testicular cancer predominantly affects men between the ages of 20 and 35. While screening for testicular cancer has the potential to reduce morbidity and mortality, the most effective screening strategy is currently unknown. Additional research is indeed necessary to determine the optimal approach for testicular cancer screening. Various factors need to be considered, including the cost-effectiveness of screening programs, the potential harms associated with screening, and the ability to detect early-stage cancers accurately. Due to the low incidence of testicular cancer and the favorable outcomes in the absence of screening, many organizations also advise avoiding testicular cancer screening.

Although all of these biomarkers have showed promise in research trials, more validation and standardization are required before they can be used in ordinary clinical practice for the detection and treatment of testicular cancer. Tailor-made therapy offers a personalized method of treating testicular cancer, and proteomic and metabolomic indicators provide insights into the metabolic and protein alterations that take place in testicular cancer cells (65). The effectiveness of testicular cancer diagnosis and treatment may be enhanced by combining these approaches.

Overall, this review offers insightful information into the genetics, genomics, proteomics, metabolomics, and customized therapeutic areas of testicular cancer research. These researches are significant milestones toward creating individualized and successful testicular cancer treatments,

despite the fact that there is still much to learn about the biology of testicular cancer. Pharmacogenomics can also help with drug selection and dosage, which will benefit cancer patients' prognoses. The management of tumors now places a high priority on genetics and pharmacogenomics.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review

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Omics sciences and precision medicine in melanoma

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Abstract

Background. This article provides an overview of the application of omics sciences in melanoma research. The name omics sciences refers to the large-scale analysis of biological molecules like DNA, RNA, proteins, and metabolites.

Methods. In the course of this review, we have adopted a focused research strategy, meticulously selecting the most pertinent and emblematic articles related to the topic. Our methodology included a systematic examination of the scientific literature to guarantee a thorough and precise synthesis of the existing sources.

Results. With the advent of high-throughput technologies, omics have become an essential tool for understanding the complexity of melanoma. In this article, we discuss the different omics approaches used in melanoma research, including genomics, transcriptomics, proteomics, and metabolomics. We also highlight the major findings and insights gained from these studies, including the identification of new therapeutic targets and the development of biomarkers for diagnosis and prognosis. Finally, we discuss the challenges and future directions in omics-based melanoma research, including the integration of multiple omics data and the development of personalized medicine approaches.

Conclusions. Overall, this article emphasizes the importance of omics science in advancing our understanding of melanoma and its potential for improving patient outcomes. ***Clin Ter 2023; 174 Suppl. 2 (6):29-36 doi: 10.7417/CT.2023.2469***

Key words: *Melanoma, omics science, genomics, metabolomics, diagnosis, precision medicine, therapy*

Introduction

During the past few decades, the incidence of cutaneous melanoma (CM) has increased, thus turning this disease from a very uncommon condition into a malignancy of increasing medical significance. Australia and New Zealand reported the greatest incidence rates, with 30 to 60 cases per 100,000 inhabitants each year (1,2). In 2017, cuticle melanoma was the fifth most prevalent malignancy among men and the sixth most prevalent among women in the United States. Moreover, 72% of all skin cancer fatalities (excluding basal-cell and squamous-cell carcinoma of the epidermis) were attributed to cutaneous melanoma. In Europe, the 5-year age-standardized relative survival for cutaneous melanoma diagnosed in 2000-2007 ranged from 74% in Eastern Europe to 87% in Western Europe (1). The Central Malignant Melanoma Registry (CMMR) has recorded over 70,000 cases of CM in Germany. At age 80, the proportion of dense melanoma significantly increases, and reaches 20% in both sexes (2).

To date, it is widely acknowledged that an individual's melanoma risk is influenced by the interplay of genetic factors and UV exposure. Epidemiological studies have identified a history of sunburns and intermittent solar exposure as risk factors for melanoma. Interestingly, eighty percent of melanomas develop in regions with intermittent sun exposure. The role of sunlight in melanoma development has been a topic of debate for decades, as its impact on the etiology of melanoma is significantly less obvious compared to nonmelanoma skin cancer (2,3). The strongest evidence

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linking UV exposure to melanoma comes from xeroderma pigmentosum, a natural genetic experiment (4).

Invasion and metastasis are the two defining characteristics of cancer, which serve as the foundation for pathologic diagnosis and staging of melanoma (4). Early detection of malignant melanoma is crucial for reducing the overall mortality associated with this disease. Large-scale screening programs, both in the United States and abroad, have proven useful in predicting high-risk patients (3).

Despite the identification of several markers and the development of algorithms for the rapid diagnosis of melanomas, tumors are often detected at an advanced stage, leading to a poor prognosis. It is widely recognized that the tumor microenvironment plays a crucial role in providing biomarkers for cancer. By analyzing markers such as lymphocyte cytosolic protein 2, autophagy, beclin 1, regulator 1, and loricrin, new insights into the role of the tumor microenvironment in melanoma progression have emerged. Furthermore, proteins like nicotinamide N-methyltransferase and TBC show promise as potential diagnostic markers for melanoma (5).

Variations in specific genes, influencing both the skin's protective response to UV light and the risk of melanoma, control how exposure to UV light affects us. Particularly, MITF amplification is more common in tumors with a poor prognosis and is connected to chemoresistant behavior. Mutant BRAF protein induces cell senescence in human melanocytes by increasing the expression of the cell-cycle inhibitor of kinase 4A (INK4A) (6).

In this article, we will discuss the epidemiology, genetic factors, and the use of omics sciences in refining diagnosis and treatment based on recent research and studies.

Genetics & Genomics

Having a family history of malignancy is linked to a higher risk of developing melanoma, as approximately 10% of melanoma cases have reported a relative with the disease. While most genetic changes related to melanoma development are somatic, the prevalence of heritable melanoma risk genes remains a critical factor in the occurrence of the disease (7-10).

High penetrance melanoma predisposition genes known to date include CDKN2A, CDK4, BAP1, POT1, ACD, TERF2IP, and TERT. Although these mutations are associated with approximately 50% of familial melanoma cases, the genetic basis for the remaining high-density melanoma families remains unidentified. The most extensively documented correlation is between CDKN2A germ line mutations and pancreatic cancer, whereas BAP1 germ line mutations have been linked to a cancer syndrome involving cutaneous melanoma, uveal melanoma, and mesothelioma. Other melanoma susceptibility genes with moderate to high penetrance have also been associated with renal cell carcinoma (MITF, BAP1) and glioma (POT1) (7,9-12).

Common gene mutations associated with melanoma

Numerous gene mutations have been linked to the development and progression of melanoma. These genes are involved in numerous signaling pathways, such as the receptor tyrosine kinase (RTK), phosphotidylinositol-3-kinase (PI(3)K), retinoblastoma (RB), p53, Wnt, and NFkB pathways (8,13).

BRAF, a serine-threonine kinase, is located in the MAP kinase signaling pathway downstream of RAS. BRAF mutations are found in approximately 60% of melanomas, and are particularly prevalent in melanomas that originate in locations with intermittent UV exposure. BRAF's oncogenic potential derives from its ability to phosphorylate MEK, which activates ERK and promotes cell proliferation. Due to their shared pathway membership, NRAS and BRAF mutations as well as NRAS and PTEN mutations are mutually exclusive in melanoma, whereas BRAF and PTEN mutations coexist in up to 20% of melanomas (8,11,13,14).

NRAS, a gene that encodes a member of the RAS family of small GTP-binding proteins, was among the first genes found to be specifically mutated in melanoma. The recurrence and high transforming potential of oncogenic NRAS mutations in human melanomas highlight the crucial role of this gene and its downstream effector mechanisms in melanoma development. NRAS mutations are the second most prevalent, affecting 20-30% of CM cases. The nodular subtype of melanoma is frequently associated with NRAS mutations that arise on the chronically UV-damaged skin of elderly patients. Additionally, NRAS-mutant melanomas tend to exhibit greater aggressiveness compared to BRAF-mutant melanomas, as evident from higher Breslow thickness and mitotic rate. (8,11,13,14).

MITF represents a novel category of lineage-survival oncogenes. Unlike oncogenic NRAS and BRAF, which gain novel and tumor-specific cellular functions through nucleotide mutations, MITF becomes oncogenic through deregulation, influencing survival mechanisms that are also present in the normal melanocyte lineage. It is widely accepted that wild-type MITF is crucial for lineage survival, and the absence (or loss) of melanocytes during development occurs in the absence of MITF (8,13).

Table 1. Common genetic mutations in melanoma.

Gene	Mutation	Frequency (%)
BRAF	V600E/K	40-50
NRAS	Q61R/K	15-20
NF1	Loss of function	10-15
KIT	L576P, K642E	2-3
TP53	Missense, truncating	1-2
CDKN2A	Loss of function	10-15
PTEN	Loss of function	5-10

Syndromes associated with melanoma

BAP1 tumor predisposition syndrome (BAP1-TPDS) is linked to an elevated risk for a particular cutaneous lesion and BAP1-inactivated melanocytic tumors. BAP1-TPDS is inherited autosomally and dominantly; currently, the majority of BAP1-TPDS patients have affected parents. First associated with BAP1-TPDS in 2011, CM is now recognized as the third most prevalent malignancy in BAP1-TPDS patients (15).

Multiple skin tumors—including cylindromas, spiradenomas, trichoepitheliomas, and (infrequently) membranous basal cell adenoma of the salivary gland—typically appear in the second or third decade in patients with CYLD cutaneous syndrome (CCS). Overall, women have more malignancies than men. Germline pathogenic variants of CYLD are autosomal dominantly inherited; the majority of people with CYLD cutaneous syndrome inherit it from their parents (16).

POT1 tumor predisposition (POT1-TPD), an autosomal dominant inheritance, is distinguished by an increased lifetime risk for multiple cutaneous melanomas, among other cancers. Currently, the majority of POT1-TPD patients have affected parents (17).

Multiple café au lait macules, intertriginous freckling, and multiple cutaneous neurofibromas characterize Neurofibromatosis 1 (NF1), a multisystem disorder. NF1 is an autosomal dominant inherited disorder, but approximately half of the affected individuals having NF1 due to *de novo* NF1 disease-causing variant. NF1-mutant tumours are aggressive and have a poor prognosis for survival; they are also prevalent in elderly patients with sun-exposed skin. NF1 mutations have been reported in roughly 14% of melanomas. Currently, there are no treatments on the market that target mutant NF1 cancers exclusively (14,18).

Proteomics, Metabolomics & Microbiomics

In the past decade, innovative molecular and proteomic analysis tools have revolutionized the discovery of cancer biomarkers. Proteomic strategies can be categorized into two groups: Those that characterize the entire protein complement of the cells or tissue of interest and those that analyze only the proteins present in specific specimens (typically blood, but also other fluids like saliva or urine) (19). More than 51,100 biomarkers have been identified and studied for melanoma. These biomarkers encompass tissue-based tumor cell and tumor microenvironment biomarkers, along with circulating tumor DNA (cf-DNA), mir-RNA, proteins, and metabolites. These biomarkers offer invaluable insights for diagnosing, prognosing, and predicting treatment response (20).

Biomarkers are used for screening (to determine who is more prone to developing multiple myeloma), diagnostics (to equip clinicians with the ability to accurately diagnose multiple myeloma), and staging (to determine the total melanoma burden present in a patient at any given time). In addition, they are used to provide information on mechanisms for combating metastatic disease and the development of novel treatments, as well as to predict and clarify the likelihood of disease progression and treatment response (20).

The comprehensive analysis of gene expression has significantly enhanced the understanding of tumorigenesis, invasion, and metastasis. Recently, gene expression assays have played a crucial role in guiding therapeutic decision-making. However, the current staging system for melanoma, which relies on Breslow thickness, ulceration, and mitotic count, proves inadequate. The varied progression rate and the presence of regional and distal metastases categorize patients into heterogeneous groups with diverse outcomes and therapeutic responses. Consequently, more aggressive surgical and adjuvant therapies are applied to large populations, leading to a diluted therapeutic effect and exposing more patients to potential toxicity (19).

Metabolite phenotyping facilitates the development of novel therapies and improves the understanding of complex metabolic diseases, such as melanoma (21,22). Single-cell omics methods have revolutionized biology by unraveling the heterogeneity that underlies population averages. One potential application is pharmaco-omics, wherein the genetic or functional makeup of diseased tissues is used to guide the implementation of personalized therapeutic strategies for patients. An example of this is Raman spectro-microscopy, which involves spatial mapping of metabolites within individual cells, aiming to identify druggable metabolic susceptibilities in a series of patient-derived melanoma cell lines (23).

Regardless of the genetic driver mutation, different ceramide and phosphatidylcholine species were observed among melanoma subtypes. Additionally, beta-alanine metabolism showed variations among melanoma subtypes and exhibited significantly higher levels in the plasma of mice with melanoma compared to healthy mice. Furthermore, beta-alanine, p-cresol sulfate, sarcosine, tiglylcarnitine, two dihexosylceramides, and phosphatidylcholine were identified as potential plasma biomarkers for melanoma (22).

Currently, immune checkpoint inhibitors (ICIs), especially antibodies targeting the cytotoxic T-lymphocyte-

Table 2. Syndromes associated with Melanoma.

Syndrome	Gene	Inheritance	Associated Cancers	Other Clinical Features
BAP1 Tumor Predisposition Syndrome	BAP1	Autosomal dominant	Uveal melanoma, cutaneous melanoma, mesothelioma, renal cell carcinoma	Atypical melanocytic lesions, ocular melanocytosis
CYLD Cutaneous Syndrome	CYLD	Autosomal dominant	Cylindromas, spiradenomas, trichoepitheliomas	Brooke-Spiegler syndrome
POT1 Tumor Predisposition	POT1	Autosomal dominant	Cutaneous melanoma, glioma, chronic lymphocytic leukemia	Familial melanoma
Neurofibromatosis 1	NF1	Autosomal dominant	Neurofibromas, optic pathway gliomas, malignant peripheral nerve sheath tumors	Café-au-lait spots, Lisch nodules, neurofibromas

associated protein 4 (CTLA-4) or the programmed death 1 (PD1) immune checkpoints, are considered the mainstay of melanoma immunotherapy. However, approximately 50% of patients do not respond to treatment (24, 25). Immunotherapies often face primary resistance, and despite initial remarkable responses to MAPK signaling inhibitors, acquired drug resistance eventually develops (26). Adoptive cell transfer (ACT) of tumor-infiltrating lymphocytes (TILs) is an alternative immunotherapeutic approach that demonstrates high efficacy in melanoma treatment (25).

Those involved in melanoma treatment are in desperate need of improved prognostic and predictive markers, but so far, these markers have remained elusive. Several tissue markers, such as S100, MART-1, and gp100/HMB45, are utilized to differentiate melanoma from other types of malignancies. Lactate dehydrogenase (LDH), which correlates with advanced-stage tumor development, stands as the most robust independent prognostic factor in stage IV melanoma and serves as the strongest prognostic serum biomarker (19).

Despite the widespread use of immunohistochemical markers, S-100 remains the most sensitive marker for melanocytic lesions, while markers like HMB-45, MART-1/Melan-A, tyrosinase, and MITF demonstrate relatively excellent specificity but not as high sensitivity as S-100. Ki67 remains the most effective adjunct for distinguishing benign melanocytic tumors from malignant ones (27).

Microbiome & Melanoma

Increasing evidence suggests that the gut microbiome is intimately associated with a variety of pathophysiological processes and plays crucial roles in antitumor immunotherapy by shaping the systemic immune response (24,28,29). Short chain fatty acids (SCFAs) and other microbiome-derived metabolites are currently recognized as mediators of tumor pathogenesis and immunotherapy. Icariside I, a novel anticancer agent isolated from *Epimedium*, inhibited B16F10 melanoma growth in vivo by modulating gastrointestinal microbiota and host immunity. Icariside I exhibited potent immunological anti-tumor activity, as indicated by the upregulation of multiple lymphocyte subsets, including CD4+ and CD8+ T cells as well as NK and NKT cells, in the peripheral blood of mice with tumors (24).

The studies presented evidence of fungi being present intratumorally and often spatially associated with cancer cells and macrophages. Comparison of intratumoral fungal communities with corresponding bacteriomes and immunomes revealed co-occurring bi-domain ecologies, frequently characterized by permissive rather than competitive microenvironments and distinct immune responses. Clinically focused evaluations suggested prognostic and diagnostic potential of tissue and plasma mycobiomes even in stage I malignancies, along with synergistic predictive performance when combined with bacteriomes (30).

Lipids & Melanoma

Among the most notable features of metabolic reprogramming is the heightened rate of lipid synthesis, which has emerged as a mediator influencing both traditional oncogenic signaling pathways and the progression of melanoma. Various alterations in fatty acid metabolism have been reported to contribute to the aggressiveness of melanoma cells. Notably, a high level of the lipogenic enzyme fatty acid synthase is associated with tumor cell invasion and poor prognosis. Fatty acid assimilation from the surrounding microenvironment, fatty acid oxidation, and fatty acid storage all appear to play a crucial role in tumor cell migration (31-33).

Pharmacogenomics

Genetic variation influences an individual's response to pharmacological treatments. Understanding this variation has the potential to improve the safety and efficacy of therapy by guiding the selection and administration of medications for a specific patient. In the context of cancer, tumours may contain mutations that define the disease, but a patient's germline genetic variation also influences drug response (both efficacy and toxicity) (34,35). In developed countries, adverse drug reactions (ADRs) are among the top 10 main causes of mortality and illness. ADRs exhibit distinct characteristics based on genotype, age, gender, race, pathology, drug class, route of administration, and drug-drug interactions. Pharmacogenomics (PGx) provides the physician with useful information for optimizing

Table 3. Melanoma Treatment and Biomarkers.

Melanoma Treatment and Prognostic Markers	Summary
Mainstay of melanoma immunotherapy	Immune checkpoint inhibitors (ICIs), particularly targeting CTLA-4 and PD-1
Treatment response	Approximately 50% of patients do not respond to ICIs
Alternative immunotherapeutic approach	Adoptive cell transfer (ACT) of tumor-infiltrating lymphocytes (TILs)
Resistance to treatment	Primary resistance to immunotherapies is common; acquired drug resistance can develop with MAPK signaling inhibitors
Prognostic markers	Lactate dehydrogenase (LDH) is the strongest independent prognostic factor in stage IV melanoma; S100 is the most sensitive marker for melanocytic lesions
Additional tissue markers	MART-1, gp100/HMB45, HMB-45, tyrosinase, and MITF have high specificity, but lower sensitivity than S-100
Effective adjunct for distinguishing benign from malignant melanocytic tumors	Ki67

drug efficacy and safety in the treatment of serious medical conditions (36,37).

Cancer subtypes may be driven by somatic mutations, or somatic mutations may merely be bystanders. When identifying somatic mutations in DNA-sequencing studies, tumour samples are a mixture of cancerous and normal cells, which must be accounted for. When investigating somatic mutations to identify a suitable targeted therapy, it is essential to consider the relevant pathways (35).

2-Hydroxyoleic acid-inserted liposomes

The inclusion of 2OHOA in liposomes notably enhanced the concentration of hydrophobic model pharmaceuticals like mitoxantrone, paclitaxel, and all-trans retinoic acid (ATRA). In vitro, the anticancer activity of liposomes incorporating ATRA and 2OHOA was significantly superior to that of conventional liposomes containing ATRA alone. In a syngeneic mouse model of B16-F10 melanoma, mice treated with ATRA-incorporated/2OHOA-inserted liposomes exhibited a significantly slower tumor growth rate compared to the control group. Immunohistochemical analyses suggested that the increased antitumor activity of ATRA-incorporated/2OHOA-inserted liposomes was at least partially due to an increase in apoptosis induction (38).

Vemurafenib

Considering the involvement of polymorphic enzymes and drug transporters in vemurafenib pharmacokinetics, genotype-based administration could prove to be an effective approach for reducing interpatient variation and optimizing patient care. The study results indicate that patients carrying variants in ABCB1 (3435C>T) or CYP3A4*22 have an elevated risk of experiencing severe vemurafenib-related toxicities. The functional effects of these polymorphisms suggest that increased systemic exposure to vemurafenib may be responsible for the observed toxicities (39).

Polyphenols

Both cancer cells and healthy cells are substantially impacted by anticancer medications. Numerous polyphenolic extracts, when taken in conjunction with standard anti-tumor medications, can contribute to the anti-proliferative effect of the drugs and substantially reduce the adverse effects. Studies have shown the protective effects of polyphenols from *Vaccinium*, *Citrus*, *Olea*, and *Cynara* against the adverse effects of four well-known chemotherapy agents, which are Cisplatin, Doxorubicin, Tamoxifen, and Paclitaxel (40).

Inhibitors

Melanoma development was inhibited by signal inhibitor to phospholipase, protein kinase C, Ca2+ release, calmodulin, and mitogen-activated protein kinase kinase 1/2. However, once melanoma had developed, only the inhibitor to mitogen-activated protein kinase kinase 1/2 significantly inhibited the proliferation of melanoma, with partial inhibition by inhibitors to protein kinase C and phospholipase C. The expression of phosphorylated extracellular signal-regulated kinase 1/2 and Ki-67 was highly correlated with the inhibition of melanoma proliferation. These findings indicate that activation of each mGluR1 signaling pathway is required for melanoma development. However, the extracellular signal-regulated kinase pathway is essential for melanoma proliferation (41).

Sodium dichloroacetate (DCA)

In recent decades, metabolism has become a defining characteristic of malignancy. It was specifically linked to immunotherapy resistance in melanoma. High glucose utilization and lactate production are shared characteristics of melanoma. Lactate significantly contributes to the acidification of the tumor microenvironment (TME) and imparts an immunosuppressive TME, which inhibits immunotherapy responses. Sodium dichloroacetate (DCA) redirects the precursor of lactate to mitochondrial metabolism, thereby preventing excessive lactate production (42). The use of oral sodium dichloroacetate (DCA) in patients with metastatic melanoma results in tumor reduction and long-term disease stability. It has been demonstrated in vitro and in vivo that DCA can serve as a cytostatic agent, without inducing apoptosis (43).

It is essential to recognize that the implementation of DCA as a standard in melanoma therapy faces numerous obstacles. Among these obstacles is the unmet need for instruments and markers to monitor and predict the response of melanoma metabolism to DCA, and to ascertain how patient-specific metabolic phenotypes influence this response (42).

Diagnosis, Treatment & Personalized Medicine

In the past decade, the field of melanoma has witnessed an unprecedented number of clinical advancements. Modern therapeutic strategies based on disease mechanisms have facilitated the transformation of disease management. Targeted approaches that predominantly inhibit the BRAF oncoprotein pathway have a high predictability of efficacy, but less than optimal response depth or duration. Immunotherapy is predominantly founded on the inhibition of one or two immune checkpoints and has a reduced predictability of response, but a higher proportion of long-lasting remissions (44-47).

The Human Genome Project and Human Proteome Project initiatives have substantially improved our comprehension of human health and disease, playing a crucial role in the ongoing move toward personalized medicine. These advancements are attributed to improved screening methods, novel therapeutic strategies, and a deeper understanding of the underlying biology of cancer. Nevertheless, cancer remains a complex and heterogeneous disease, subject to

modulation over time by various factors, including genetic, molecular, cellular, tissue, population, environmental, and socioeconomic influences (48).

Furthermore, genetic analysis is now playing an increasingly significant role in guiding patient care. As new genes are discovered and key molecular pathways in melanoma progression are elucidated, therapeutic interventions targeting these pathways are becoming accessible (49, 50). The advent of next-generation sequencing (NGS) technologies has made it possible to sequence multiple cancer-driving genes in a single assay, with enhanced sensitivity for mutation detection (51).

Mass spectrometry remains the principal platform for proteomics analysis, with shotgun proteomics or bottom-up being the most frequently used approach. Recently, chromatographic methods have gained widespread recognition as methodologies worthy of consideration due to their distinct advantages, particularly in sample manipulation, recovery, and automation. Multidimensional purification has been found to be particularly effective, resulting in high purification factors and reducing sample complexity before MS analysis, thus facilitating a more comprehensive exploration of the proteome (48).

The existing therapeutic approaches for melanoma include surgical resection, chemotherapy, photodynamic therapy, immunotherapy, biochemotherapy, and targeted therapy. Depending on the patient's health and tumor characteristics, treatment strategies may involve single agents or combinations of therapies. However, the effectiveness of these treatments may be reduced due to the emergence of various resistance mechanisms. Studies focusing on the genetic profile of melanocytes and the identification of molecular factors involved in the development of malignant transformation have revealed novel therapeutic targets (52,53).

Caution should be exercised when administering radiation therapy, as the combination of BRAF inhibitors and radiation therapy has been linked to increased toxicity. Patients with stage 4 melanoma, whether untreated or treated with BRAF inhibitors and MEK inhibitors, exhibit a median overall survival of 22 to 25 months, with a 3 to 5-year overall survival rate reaching 40 percent. Favorable prognostic factors include normal lactate dehydrogenase concentrations, fewer than three metastatic sites, and satisfactory Eastern Cooperative Oncology Group performance. However, a significant drawback of targeted therapy is the development of resistance during treatment (1,54).

In the context of treating metastatic melanoma, numerous novel medications have been developed in the last decade, substantially improving the prognosis for patients with this condition. However, the majority of patients do not demonstrate a long-lasting response to these treatments. As a result, new biomarkers and drug targets are needed to enhance the diagnostic and therapeutic accuracy of melanoma (55).

Conclusion

In conclusion, "omics" science has emerged as a powerful tool in the study of melanoma. The integration of genomics, proteomics, and metabolomics has allowed for a more comprehensive understanding of the molecular mechanisms involved in the development and progression of this deadly disease. "Omics" approaches have identified numerous potential biomarkers for early detection, prognosis, and treatment response, which could greatly improve patient outcomes. However, further research is needed to validate these biomarkers and translate them into clinical practice. The application of "omics" technologies in melanoma research is a promising path for the development of personalized and targeted therapies, ultimately leading to better outcomes for patients.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Omics sciences and precision medicine in lung cancer

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Abstract

Lung cancer is a complex disease, with a wide range of genetic alterations and clinical presentations. Understanding the natural and clinical history of the disease is crucial for developing effective diagnostic and treatment strategies. Omics approaches, such as genomics, transcriptomics, proteomics, and metabolomics, have emerged as powerful tools for understanding the molecular mechanisms underlying lung cancer and for identifying novel biomarkers and therapeutic targets. These approaches enable researchers to examine the entire genome, transcriptome, proteome, or metabolome of a cell or tissue, providing a comprehensive view of the biological processes involved in lung cancer development and progression. Targeted therapies that address specific genetic mutations and pathways hold promise for improving the diagnosis and treatment of this disease. ***Clin Ter 2023; 174 Suppl. 2 (6):37-45 doi: 10.7417/CT.2023.2470***

Key words: *Lung cancer, tumor, genetics, genomics, pharmacogenomics, tailored therapy.*

Introduction

Lung cancer is a complex disease with a multifactorial etiology, involving a combination of genetic and environmental factors. The understanding of tumor natural and clinical history, as well as its genetics, has significantly improved in recent years, leading to the development of new diagnostic and therapeutic approaches. Genetic testing can identify predisposition to lung cancer and provide valuable information for tailoring treatment options. Additionally, the genomics of the tumor, including rearrangements, fusion genes, and somatic mutations, can guide targeted therapies (1, 2). Other emerging biomarkers—such as circulating tumor DNA, proteomic, metabolomic, and microbiomic profiling—may provide further insights into the pathogenesis and personalized management of lung cancer (2).

In recent years, the role of genomics in tumor management has expanded rapidly. Chromosomal rearrangements, fusion genes, and somatic mutations are common genetic alterations that occur in cancer and can be identified through genomic analysis (3). Chromosomal rearrangements, such as translocations or deletions, are frequent genetic events in cancer, which can lead to altered expression or function of the genes involved in cell proliferation, differentiation, and apoptosis, resulting in cancer development and progression (4). Fusion genes, formed as a result of chromosomal rearrangements, play a significant role in the development of cancer by activating or inactivating pathways involved in cell growth and survival (5). Somatic mutations, which are genetic alterations that occur in non-germline cells, can also affect genes involved in cellular processes and contribute to the development of cancer (6).

The identification of specific genetic alterations in tumors has led to the development of targeted therapies that aim to block the signaling pathways affected by these alterations. The use of targeted therapies has significantly improved the outcomes of cancer treatment for some patients, particularly those with specific genetic alterations that are targeted by the therapy (7). One example of this is the use of tyrosine kinase inhibitors to target the BCR-ABL fusion protein in chronic myeloid leukemia (8).

Another area of genomics in tumor management that has gained significant attention is the use of liquid biopsies,

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which involve the analysis of circulating tumor cells or circulating tumor DNA (ctDNA) in the blood. ctDNA analysis has emerged as a promising biomarker for cancer diagnosis and prognosis, and for monitoring treatment response (9). It can provide information on the genetic alterations present in the tumor, such as somatic mutations and chromosomal rearrangements, which can guide the selection of targeted therapies (9).

Pharmacogenomics—the study of genetic variations that influence drug response and toxicity—is also playing an increasingly important role in tumor management. The identification of genetic polymorphisms that affect drug metabolism, transport, and pharmacodynamics has the potential to improve drug efficacy and safety. Moreover, pharmacogenomics can inform drug selection and dosing, which can improve treatment outcomes for cancer patients (10).

Genomics and pharmacogenomics have become the forefront of tumor management. Advances in technology have made it possible to identify specific genetic alterations in tumors and to develop targeted therapies that can improve treatment outcomes. The use of liquid biopsies and pharmacogenomics has also emerged as promising approaches for cancer diagnosis, prognosis, and treatment selection. The integration of genomics and pharmacogenomics into clinical practice has the potential to improve personalized cancer treatment and ultimately improve patient outcomes.

Lung Tumor natural history and clinical history

Lung cancer is a complex disease, with a natural history that involves its progression from initial formation to metastatic spread. The clinical history of lung cancer, on the other hand, focuses on the symptoms and physical manifestations of the disease in the patient. A better understanding of the natural and clinical history of lung cancer is crucial for developing effective diagnostic and treatment strategies.

According to a study by Seijo et al. (2019), the natural history of lung cancer is determined by the interaction between the tumor and the host. The authors describe the process of tumor development, from the initial genetic mutations to the formation of preneoplastic lesions, and the subsequent progression to invasive cancer. The authors also discuss the role of the immune system in tumor development and progression (11).

In terms of clinical history, the symptoms of lung cancer vary depending on the stage and location of the tumor. According to the American Cancer Society (2021), common symptoms of lung cancer include persistent cough, chest pain, shortness of breath, hoarseness, weight loss, and coughing up blood. However, many patients with lung cancer may not experience any symptoms until the disease has progressed to an advanced stage (12).

Lung cancer is a complex disease that involves multiple genetic and environmental factors. The natural history of lung cancer is characterized by the progressive accumulation of genetic alterations that ultimately result in the development of a malignant tumor (13). The development of lung cancer can be divided into several stages, including initiation, promotion, and progression. The initiation stage involves exposure to carcinogens, such as tobacco smoke, which causes DNA damage in lung cells (14). The promotion stage involves the clonal expansion of mutated cells, which can eventually lead to the development of a preneoplastic lesion (15). Finally, the progression stage involves the acquisition of additional genetic alterations, which allow the preneoplastic lesion to progress to an invasive carcinoma (16).

The clinical history of lung cancer is often characterized by a long asymptomatic phase followed by the onset of symptoms, which can vary depending on the location and size of the tumor. Symptoms of lung cancer may include cough, chest pain, shortness of breath, hemoptysis, weight loss, and fatigue. However, many patients may not experience symptoms until the cancer has reached an advanced stage (17). Thus, early detection of lung cancer is crucial for improving patient outcomes.

Several risk factors have been identified for the development of lung cancer, including smoking, exposure to radon gas, air pollution, and occupational exposure to carcinogens such as asbestos and diesel exhaust. Smoking remains the most significant risk factor for lung cancer, accounting for approximately 85% of cases (14). As such, smoking cessation is the most effective way to reduce the risk of developing lung cancer.

Genetics of the tumor

Genetic alterations play a significant role in the development and progression of lung cancer. Mutations in oncogenes, tumor suppressor genes, and DNA repair genes have been frequently found in lung cancer. The identification of these genetic alterations has led to the development of targeted therapies for lung cancer. For example, EGFR mutations are commonly found in non-small cell lung cancer (NSCLC) patients, and drugs targeting these mutations have been developed, such as gefitinib and erlotinib. Other mutations, such as ALK, ROS1, and BRAF, have also been identified as actionable targets in NSCLC (18).

Genetics has undergone significant advancements over the past few decades, which has led to the discovery of numerous genetic variations that influence tumor development and progression. Genetic testing has become an essential component in tumor management, as it helps in identifying individuals who are predisposed to developing certain types of tumors. Several studies have identified the association between specific genetic mutations and an increased risk of tumor development. For instance, BRCA1 and BRCA2 mutations are associated with an increased risk of breast and ovarian cancer (19, 20). Lynch syndrome, caused by mutations in DNA mismatch repair genes, is associated with an increased risk of colorectal, endometrial, and other cancers (21).

In addition to identifying germline mutations, genetic testing can also identify somatic mutations in the tumor tissue. Somatic mutations occur in non-germline cells and are not inherited. These mutations are unique to the tumor tissue and can provide information about the tumor's biology, behavior, and potential response to treatment. The identification of somatic mutations has led to the development of targeted therapies that can specifically target these mutations. For example, HER2-positive breast cancers can be treated with

trastuzumab, a targeted therapy that specifically targets the HER2 protein (22). Similarly, vemurafenib, a targeted therapy, is effective in treating melanoma patients with the BRAF V600E mutation (23). Lung cancer genetic mutations and their phenotype are reported in table 1.

Genetic Testing

Lung cancer is a complex disease that can be caused by a combination of genetic and environmental factors. Genetic testing can provide important information about a person's risk for developing lung cancer and may help guide personalized treatment options. One gene that has been implicated in the development of lung cancer is the epidermal growth factor receptor (EGFR) gene. Mutations in this gene have been found in a subset of non-small cell lung cancers (NSCLC) and are associated with a higher response rate to targeted therapy with EGFR inhibitors. Another gene that has been linked to lung cancer is the KRAS gene. Mutations in this gene are common in lung adenocarcinomas and have been associated with a poorer response to therapy. Genetic testing for these and other genes can be performed on tumor tissue samples using techniques such as next-generation sequencing. In some cases, testing may also be performed on blood samples to detect inherited genetic mutations that increase the risk of developing lung cancer (24).

It is important to note that while genetic testing can provide valuable information, it is not a substitute for regular cancer screenings or other preventative measures, such as smoking cessation. According to a study by Wu et al. (25), genetic testing can help identify patients with metastatic cancer who may benefit from targeted therapy. The study found that patients with lung cancer who had an EGFR mutation had a better response to EGFR inhibitors than those without the mutation. Similarly, another study by Peng et al. (26) reported that genetic testing can help identify patients with pancreatic cancer who may benefit from PARP inhibitors. The study found that patients with BRCA1/2 mutations had a better response to PARP inhibitors than those without the mutation.

Predisposition

Lung cancer is a multifactorial disease influenced by a complex interplay of genetic, environmental, and lifestyle factors. Environmental factors such as smoking and exposure to pollutants are known to increase the risk of lung cancer, which is why, in order to reduce the burden of lung cancer, prevention efforts aim at reducing tobacco use (the cornerstone of lung cancer prevention) and developing strategies to reduce exposure to environmental carcinogens and promote healthy lifestyle choices. However, genetics also play an important role in predisposing individuals to the disease: several genes have been identified as potential contributors to lung cancer susceptibility—including EGFR, KRAS, and TP53. Studies have shown that certain genetic variations in these genes may increase an individual's risk of developing lung cancer (27).

One of the most well-known genes associated with lung cancer is EGFR, which encodes for the epidermal growth factor receptor. Mutations in this gene have been found in a subset of non-small cell lung cancer (NSCLC) cases, particularly in patients who have never smoked. These mutations lead to increased activation of the EGFR pathway, which promotes tumor growth and survival. Other genes that are commonly mutated in NSCLC include KRAS, which is

Table 1. Lung cancer Genetic Mutations and their phenotype.

S.no	Gene	Inheritance	Phenotype	OMIM
1	FASLG	AD, Somatic Mutation	Lung cancer, susceptibility to	134638
2	CASP8		Hepatocellular carcinoma, somatic	601763
3		AD, Somatic Mutation	Lung cancer, protection against	211980
4	PIK3CA		Hepatocellular carcinoma, somatic	171834
5			Non-small cell lung cancer, somatic	211980
6	IRF1		Non-small cell lung cancer, somatic	147575
7	PRKN		Adenocarcinoma of lung, somatic	602544
8	EGFR	AD, Somatic Mutation	Adenocarcinoma of lung, response to tyrosine kinase inhibitor in	131550
9		AD, Somatic Mutation	Non-small cell lung cancer, response to tyrosine kinase inhibitor in	211980
10		AD, Somatic Mutation	Non-small cell lung cancer, susceptibility to	211980
11	BRAF		Adenocarcinoma of lung, somatic	164757
12			Non-small cell lung cancer, somatic	211980
13	MAP3K8		Lung cancer, somatic	191195
14	ERCC6	AD, Somatic Mutation	Lung cancer, susceptibility to	609413
15	SLC22A1L		Lung cancer, somatic	602631
16	PPP2R1B		Lung cancer, somatic	603113
17	KRAS		Lung cancer, somatic	190070
18	ERBB2		Adenocarcinoma of lung, somatic	164870
19	CYP2A6	AD, Somatic Mutation	Lung cancer, resistance to	122720

involved in cell signaling pathways, and TP53, which plays a key role in regulating cell growth and preventing cancer (28). While genetic predisposition to lung cancer is not fully understood, several risk factors have been identified. For example, individuals with a family history of lung cancer are more likely to develop the disease themselves. Additionally, certain ethnic groups, such as individuals of Asian descent, have a higher prevalence of EGFR mutations and are more likely to develop lung cancer at a younger age (29).

Correlated Syndromes

Correlated syndromes in lung cancer refer to the occurrence of multiple clinical manifestations that may be associated with lung cancer, but not necessarily are caused by the tumor itself. For example, paraneoplastic syndromes, which are caused by immune-mediated reactions to the tumor, can result in a variety of symptoms like neurological deficits, endocrine abnormalities, and dermatological manifestations. Additionally, patients with lung cancer may experience chronic obstructive pulmonary disease (COPD), a condition characterized by chronic inflammation and narrowing of the airways, which can exacerbate respiratory symptoms and decrease lung function. Furthermore, lung cancer is also associated with an increased risk of developing venous thromboembolism (VTE), a potentially life-threatening condition that involves blood clots forming in the veins of the legs or lungs. The presence of these correlated syndromes can complicate the diagnosis and treatment of lung cancer and may require a multidisciplinary approach to management (30).

In addition to genetic testing, several studies have also emphasized the importance of identifying clinical syndromes to provide appropriate preventive measures. For example, a study by Saidane et al. (31) found that families with a history of lung cancer have an increased risk of developing the disease, even in the absence of smoking, thus identifying the familial lung cancer syndrome. This knowledge allows clinicians to provide tailored screening recommendations to detect lung cancer at an early stage, potentially reducing mortality. Similarly, the identification of the Lynch syndrome, as reported by Lee et al. (32), allows for tailored screening recommendations and risk-reducing interventions for individuals at increased risk of colorectal and other cancers.

The identification of these correlations helps in identifying individuals with a predisposition to tumor development and selecting appropriate screening and management strategies. The paper by Bonadona et al. (33) discusses the importance of identifying individuals with Lynch syndrome, as these individuals have an increased risk of colon cancer, while the paper by Gueye Tall et al. (34) discusses the correlation between specific genetic mutations and the risk of developing Wilms tumor in individuals with certain tumor syndromes.

Overall, the identification of correlated syndromes and genetic mutations through testing is critical in the management of hereditary cancer syndromes: it allows for tailored surveillance, risk-reducing interventions, and enhanced screening measures to detect cancer at an early stage. With the increasing availability and affordability of genetic testing, it is essential to incorporate genetic testing into clinical practice to provide optimal care to individuals at risk of developing hereditary cancer syndromes.

Genomics of the tumor

Cancer is a heterogeneous disease with diverse genetic alterations. Advances in genomics have enabled the identification of somatic mutations, fusion genes, and chromosomal rearrangements in various cancer types. The identification of driver mutations that cause oncogenic transformation has facilitated the development of targeted therapies for cancer patients. Several studies have reported genetic aberrations in different types of tumors, and these findings have improved understanding and management of these cancers.

Genetic alterations are common in lung cancer and can contribute to tumor development and progression. A variety of genetic mutations have been identified in lung cancer, including mutations in the EGFR, KRAS, and TP53 genes. EGFR mutations, in particular, are associated with a subset of non-small cell lung cancers (NSCLC) and can be targeted by certain drugs, such as EGFR tyrosine kinase inhibitors (TKIs). Other mutations, such as KRAS and TP53, are associated with a more aggressive form of NSCLC and are less responsive to targeted therapies. Understanding the genetic makeup of lung tumors can help guide treatment decisions and improve outcomes for patients (35).

Rearrangements

Chromosomal rearrangements are common in lung cancer and can result in the activation of oncogenes or the inactivation of tumor suppressor genes. One well-known example is the EML4-ALK fusion gene, which is found in about 5% of non-small cell lung cancer cases. This fusion gene results from a chromosomal inversion that brings together the EML4 gene on chromosome 2 and the ALK gene on chromosome 2. This fusion leads to constitutive activation of the ALK protein, which can drive tumor growth and survival. Other chromosomal rearrangements that have been identified in lung cancer include the RET fusions, ROS1 fusions, and NTRK fusions (36).

In addition to the EML4-ALK fusion gene, numerous other chromosomal rearrangements were identified in lung cancer. For example, RET fusions result from a fusion between the RET gene and various other partner genes. These rearrangements are found in approximately 1-2% of non-small cell lung cancer cases and can lead to constitutive activation of the RET protein, which plays a role in cell growth and survival. Similarly, ROS1 fusions and NTRK fusions have also been identified in a subset of lung cancers and can result in the activation of the ROS1 and NTRK proteins, respectively (37). The identification of these chromosomal rearrangements has led to the development of targeted therapies for lung cancer. For example, crizotinib is a drug that targets ALK, ROS1, and MET, and has been shown to be effective in patients with EML4-ALK or ROS1 fusions. Similarly, drugs targeting RET and NTRK are currently under development and have shown promise in early clinical trials (38).

Overall, chromosomal rearrangements play an important role in the development of lung cancer and have provided new targets for the development of targeted therapies. Further research is needed to fully understand the role of these rearrangements in lung cancer and to develop more effective treatments for this devastating disease.

Fusion genes

Fusion genes are common in lung cancer and result from chromosomal rearrangements that bring together two previously separate genes, resulting in a hybrid gene. These fusion genes can drive tumorigenesis by altering normal cellular functions, such as signal transduction, gene expression, and cell cycle regulation. One well-known example of a fusion gene in lung cancer is the EML4-ALK fusion gene, which is found in a subset of non-small cell lung cancer cases. The fusion gene results from an inversion of the short arm of chromosome 2, leading to the fusion of the EML4 gene with the ALK gene. This fusion results in the constitutive activation of the ALK protein, leading to increased cell proliferation and survival (39).

In addition to the EML4-ALK fusion gene, other fusion genes have also been identified in lung cancer. For example, the ROS1 fusion gene, which results from rearrangements involving the ROS1 gene, is found in approximately 1-2% of non-small cell lung cancer cases. This fusion gene leads to the constitutive activation of the ROS1 protein, which can drive tumor growth and survival. Other fusion genes that have been identified in lung cancer include RET fusions, NTRK fusions, and MET fusions (40).

The identification of fusion genes in lung cancer has led to the development of targeted therapies that specifically target these genetic abnormalities. For example, crizotinib—a drug targeting ALK, ROS1, and MET—has been shown to be effective in patients with EML4-ALK or ROS1 fusions. Similarly, drugs targeting RET and NTRK fusions are currently under development and have shown promise in early clinical trials (41).

Fusion genes play an important role in the development of lung cancer, and their identification has provided new targets for the development of targeted therapies. Further research is needed to fully understand the role of fusion genes in lung cancer and to develop more effective treatments for this devastating disease.

Somatic mutations

Somatic mutations in lung cancer have been widely studied, as they are thought to play a crucial role in the development and progression of the disease. One of the most frequently mutated genes in lung cancer is TP53, which encodes for the tumor suppressor protein p53. In a study by Vogelstein et al. (2013), it was found that TP53 mutations were present in 50-70% of lung adenocarcinomas and were associated with poorer overall survival. Another commonly mutated gene in lung cancer is EGFR, which encodes for the epidermal growth factor receptor (42). In a study by Paez et al. (2004), EGFR mutations were found associated with increased sensitivity to tyrosine kinase inhibitors. Other frequently mutated genes in lung cancer, among others, include KRAS, BRAF, and ALK. Overall, these studies highlight the importance of somatic mutations in lung cancer, both as prognostic markers and as potential targets for therapy (43). Several studies have also shown that somatic mutations in genes such as TP53, KRAS, and EGFR are common in lung cancer patients. The identification of these somatic mutations has led to the development of targeted therapies that can selectively inhibit the growth of cancer cells. For example, EGFR mutations have been found in about 10-15% of non-small cell lung cancer (NSCLC) patients, and drugs targeting these mutations, such as gefitinib and erlotinib, have been developed and approved for clinical use. Other mutations, such as those in ALK, ROS1, and BRAF, have also been identified as actionable targets in NSCLC (44).

Circulating tumor

Circulating tumor DNA (ctDNA) is fragmented DNA released into the bloodstream by tumor cells undergoing apoptosis or necrosis, which has emerged as a promising biomarker for lung cancer diagnosis and monitoring. In fact, ctDNA analysis through liquid biopsy offers a non-invasive approach to detect and track disease progression. Several studies have also shown the potential of ctDNA in identifying genetic alterations like EGFR mutations and ALK rearrangements in non-small cell lung cancer (NSCLC) patients, which can guide targeted therapy selection and improve patient outcomes (45).

In addition to its potential for diagnosis and monitoring of lung cancer, ctDNA also showed promise in predicting treatment response and disease recurrence. Studies have demonstrated that ctDNA levels can be used as a predictor of response to therapy, with patients who experience a reduction in ctDNA after treatment having a higher likelihood of a positive treatment response (46). Furthermore, ctDNA analysis has the potential to detect minimal residual disease (MRD), which refers to the presence of small amounts of cancer cells that remain in the body after treatment and can lead to disease recurrence (47). By monitoring ctDNA levels over time, clinicians can identify patients at high risk of recurrence and adjust treatment accordingly, thus potentially improving patient outcomes. Overall, the use of ctDNA as a biomarker for lung cancer holds great promise for improving both diagnosis and treatment strategies, ultimately leading to better patient outcomes.

Pharmacogenomics

Pharmacogenomics is the study of genetic variations that influence drug response and toxicity. The identification of genetic polymorphisms that affect drug metabolism, transport, and pharmacodynamics has the potential to improve drug efficacy and safety. Moreover, pharmacogenomics can also inform drug selection and dosing, leading to personalized treatment options. In lung cancer treatment, pharmacogenomics can help identify patients who are likely to experience adverse drug reactions or who may not respond to certain therapies due to their genetic makeup. Several genetic variations, including those in the EGFR and ALK

genes, have been identified as predictive biomarkers for targeted therapy in non-small cell lung cancer (NSCLC) patients. Moreover, pharmacogenomics-guided dosing of chemotherapy drugs has been shown to improve patient outcomes by reducing toxicity and improving efficacy (48). For instance, in breast cancer, the presence of CYP2D6 polymorphisms is associated with altered metabolism of tamoxifen, a drug commonly used in the treatment of estrogen receptor-positive breast cancer. Patients with reduced CYP2D6 activity may have a lower response to tamoxifen and a higher risk of recurrence (49). Similarly, in colorectal cancer, the presence of KRAS mutations is associated with resistance to anti-EGFR therapy (50). Therefore, the use of pharmacogenomic testing can identify patients who are likely to benefit from a particular treatment and avoid the use of ineffective or harmful therapies in others.

Plasma and tissue proteomic biomarkers

Lung cancer is a highly aggressive and heterogeneous disease that often manifests non-specific symptoms, thus leading to late diagnosis and poor prognosis. Therefore, it is critical to identify non-invasive biomarkers that can improve the diagnosis and prognosis of lung cancer.

Plasma and tissue proteomics have emerged as promising tools for the discovery and validation of lung cancer biomarkers. Plasma proteomics enables the identification of circulating proteins that reflect the pathophysiological changes associated with lung cancer, while tissue proteomics provides insights into the molecular mechanisms underlying lung cancer development and progression. Several studies have demonstrated the potential of plasma and tissue proteomic biomarkers for lung cancer diagnosis, prognosis, and treatment monitoring (51, 52). For instance, a study by Chen et al. (2019) identified a panel of plasma proteins that could distinguish lung cancer patients from healthy controls with high sensitivity and specificity (53). Similarly, a study by Tang et al. (2018) identified tissue proteomic biomarkers that were associated with lung cancer prognosis and response to treatment (54). Overall, plasma and tissue proteomics represent valuable approaches for the identification and validation of lung cancer biomarkers, which have the potential to improve the management and outcomes of lung cancer patients.

Plasma and tissue metabolomics and microbiomics

As mentioned above, genetic predisposition aside, lung cancer is also influenced by environmental factors. Recent advances in technology have enabled the study of lung cancer using metabolomics and microbiomics approaches. Metabolomics is the study of small molecules, such as metabolites, that are produced by cells and tissues, while microbiomics focuses on the study of the microbial communities that inhabit different environments. In the context of lung cancer, plasma and tissue metabolomics and microbiomics have been used to identify potential biomarkers for early detection, prognosis, and treatment response.

Several studies have investigated the role of plasma metabolomics in lung cancer. For example, a study by Li et al. (2020) used liquid chromatography-mass spectrometry (LC-MS) to analyze the plasma metabolome of lung cancer patients and healthy controls. The study identified a panel of metabolites that could distinguish between lung cancer patients and controls with high accuracy. Similarly, tissue metabolomics has also been used to identify potential biomarkers for lung cancer (55). A study by Wang, H., et al 2018 used gas chromatography-mass spectrometry (GC-MS) to analyze the metabolome of lung cancer tissues and adjacent normal tissues. The study identified several metabolites that were significantly altered in lung cancer tissues, including amino acids and fatty acids. Microbiomics has also been studied in the context of lung cancer (56). A study by Jin et al. (2020) used 16S rRNA gene sequencing to analyze the lung microbiome of lung cancer patients and healthy controls. The study found that the composition of the lung microbiome was significantly different between lung cancer patients and controls, and that certain microbial taxa were associated with better or worse prognosis (57). Another study by Jin et al. (2021) used metagenomic sequencing to analyze the lung microbiome of lung cancer patients before and after treatment with immune checkpoint inhibitors. The study found that changes in the lung microbiome were associated with treatment response, suggesting that the lung microbiome may be a potential biomarker for predicting treatment response (26).

Plasma and tissue metabolomics and microbiomics are valuable tools for studying lung cancer. These approaches have the potential to identify new biomarkers for early detection, prognosis, and treatment response, and to improve our understanding of the underlying biology of this complex disease. Metabolites and biomarkers for lung cancer are reported in Table 2.

It's worth noting that this is not an exhaustive list, and there are many other metabolites and biomarkers that may be relevant for lung cancer diagnosis and prognosis. Additionally, the sensitivity and specificity of these markers may vary depending on the specific context and population being studied (58, 59).

Personalized and Tailored therapy

Personalized and tailored therapy is an emerging approach to cancer treatment that aims to provide individualized treatment plans based on the patient's unique molecular and genetic characteristics. This approach has shown great promise in the treatment of lung cancer, as it allows for more targeted and effective therapies that are tailored to the individual patient's needs (60).

One example of personalized therapy for lung cancer is the use of molecular testing to identify specific genetic mutations or abnormalities in the cancer cells. These mutations can be targeted with specific drugs that block the growth and spread of the cancer cells. For example, the drug crizotinib targets the ALK gene mutation, which is found in about 5% of non-small cell lung cancers. Similarly, the drug osimertinib targets the EGFR gene mutation, which is found in about 10% of non-small cell lung cancers (61).

Table 2. Metabolites and biomarkers for lung cancer.

Metabolite/Biomarker	Type	Importance
CEA	Biomarker	Elevated levels associated with lung cancer diagnosis and prognosis
Cyfra 21-1	Biomarker	Elevated levels associated with lung cancer diagnosis and prognosis
miRNA-21	Biomarker	Overexpression associated with lung cancer development and progression
miRNA-126	Biomarker	Downregulation associated with lung cancer development and poor prognosis
NSE	Biomarker	Elevated levels associated with small cell lung cancer diagnosis and prognosis
LDH	Biomarker	Elevated levels associated with advanced stage lung cancer and poor prognosis
Glutathione	Metabolite	Decreased levels associated with lung cancer development and progression
Tryptophan	Metabolite	Decreased levels associated with lung cancer development and poor prognosis
Proline	Metabolite	Increased levels associated with lung cancer development and progression

In addition to molecular testing, other factors such as the patient's age, overall health, and other medical conditions can also be considered when developing a personalized treatment plan. For example, elderly patients or those with other medical conditions may not be candidates for aggressive chemotherapy or surgery and may benefit more from less invasive treatments such as radiation therapy or targeted therapy. Personalized and tailored therapy for lung cancer is a rapidly evolving field, with new discoveries and treatments being developed all the time. As more is learned about the genetic and molecular characteristics of lung cancer, it is likely that even more targeted and effective therapies will be developed. This approach has the potential to revolutionize the treatment of lung cancer, allowing for more precise and effective treatment plans that improve outcomes and quality of life for patients (62).

Several studies have shown the benefits of tailored therapy in cancer treatment. For example, a study on non-small cell lung cancer patients found that those who received tailored therapy had a higher response rate and longer progression-free survival than those who received standard therapy (63). Another study found that breast cancer patients who received targeted therapy based on their tumor's genetic profile had better overall survival than those who received standard therapy (64).

Conclusions

In conclusion, the natural history and clinical history of lung cancer, along with its genetic and genomic profile, provide valuable information for its diagnosis, prognosis, and treatment. Genetic testing and predisposition analysis can identify individuals who are at a higher risk of developing lung cancer. Circulating tumor DNA analysis and pharmacogenomics can help determine the best treatment strategy for each lung cancer patient. Proteomic and metabolomic biomarkers offer insights into the metabolic and protein changes that occur in lung cancer cells, and tailored therapy provides a personalized approach to lung cancer treatment. The integration of these approaches has the potential to improve lung cancer diagnosis and treatment outcomes.

Overall, the research papers included in this review provide valuable insights into the various aspects of lung cancer research, including genetics, genomics, proteomics, metabolomics, and tailored therapy. While there is still much to learn about lung cancer biology, these studies represent important steps towards developing personalized and effective lung cancer treatments.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Omics sciences and precision medicine in kidney cancer

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Abstract

In the last decade, renal carcinoma has become more prevalent in European and North American regions. Kidney tumors are usually categorized based on histological features, with renal cell carcinoma being the most common subtype in adults. Despite conventional diagnostic and therapeutic strategies, a rise in cancer incidence and recurrence necessitates a fresh approach to diagnosing and treating kidney cancer. This review focuses on novel multi-omics approaches, such as genomics, transcriptomics, proteomics, metabolomics, and microbiomics, to better understand the molecular and clinical features of renal cell carcinoma. Studies integrating omics sciences have shown early promise in enhancing prognostic and therapeutic outcomes for various kidney cancer subtypes and providing insight into fundamental pathophysiological mechanisms occurring at different molecular levels. This review highlights the importance of utilizing omics sciences as a revolutionary concept in diagnostics and therapeutics and the clinical implications of renal cell carcinoma. Finally, the review presents the most recent findings from large-scale multi-omics studies on renal cell carcinoma and its associations with patient subtyping and drug development. ***Clin Ter 2023; 174 Suppl. 2 (6):46-54 doi: 10.7417/CT.2023.2471***

Key words: *Kidney cancer, renal cell carcinoma, omics sciences, genetics, metabolomics*

Introduction

Every year, nearly 300,000 people are diagnosed with renal cell carcinoma (RCC) worldwide, with a mortality rate reaching 100,000/year (1). Geographically, Europe and North America have a higher incidence rate than Asia and South America. However, RCC's prevalence has increased by 20% in the last decade, particularly in industrialized countries (2).

RCC can be sporadic or hereditary, but it is linked to structural mutations on chromosome 3p.21 in both cases (3); usually, men are more vulnerable to RCC than women. In children, Wilms tumor is the most frequently diagnosed kidney cancer subtype, whereas in adults the most prevalent subtype malignant is the tumor of renal parenchyma adenocarcinoma cell (renal cell cancer) (2). Clear cell renal cell carcinoma (65-70%), papillary renal cell carcinoma (18-20%), chromophobe RCC (5-7%), and collecting duct carcinoma (1-2%) are the most diagnosed RCCs (4, 5). Despite reliable diagnostic and remedial approaches, the mortality rate of RCC remains too high, and in most cases cancer relapse happens, due to tumor heterogeneity (6, 7). Therefore, the real goal of this study is developing novel curative strategies, particularly for treating advanced-stage RCC patients and integrating multi-omics approaches to better understand biomarker discovery and for hastening the adoption of precision oncology in RCC. This review proposes the use of omics sciences as a potential and promising solution in RCC early diagnosis and treatment.

Kidney Cancer Diagnosis and Therapy

The conventional diagnostic methods for renal carcinoma include blood and urine tests, imaging tests (ultrasound, X-ray, CT, or MRI), and biopsy. If the patient is positive, they are usually treated with surgery, targeted therapy (Sorafenib and Sunitinib), immunotherapy, ablation, or a combination of these treatments (8). Radio- and chemotherapy are occasionally used. Metastatic kidney cancer patients frequently receive multiple lines of treatment (9).

However, these techniques have limitations, such as financial constraints, invasiveness, and the generation of false positive results during imaging testing. Furthermore,

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surgery is not appropriate for metastatic tumors, radiation may harm healthy cells, and cells may develop resistance to targeted therapy. These techniques are also time-consuming, labor-intensive, and not cost-effective (10).

So far, the potential anti-carcinogenic effects of dichloroacetate (DCA), such as inhibiting cellular proliferation, inducing apoptosis, and reducing lactate levels, make it a reassuring drug candidate against renal adenocarcinomas. Dichloroacetate salts selectively target cancer cells, causing them to switch from glycolysis to oxidative phosphorylation (11). Pyruvate delivery into mitochondria causes organelle remodeling, which leads to increased efflux of apoptosis-inducing factors and upregulation of ROS levels, resulting in a reduction in cancer cell viability (12).

The failure of conventional strategies made researchers think of effective and novel methods to address the current limitations associated with diagnosing and treating kidney cancer. The revolutionizing concept of using omics sciences for the following purpose was groundbreaking in diagnostics and therapeutics. For instance, genomic and sequencing studies have identified VHL, SETD2, and BAP1 as the most frequently studied RCC driver genes (13)—for which the mutation prevalence was 64%, 20%, and 13%, respectively—and are involved in RCC pathogenesis (14). These genes' expression profiles can determine their role as potential biomarkers for kidney cancer diagnosis. Furthermore, seven drugs targeting the VHL pathway for the treatment of patients with advanced kidney cancer have been approved by FDA. Further genomic research can lay the groundwork for better prognostic and therapeutic strategies to combat this disease (15).

Transcriptomics profiling has further highlighted the importance of omics sciences in scientific research, by providing valuable insight into identifying biomarkers and kidney cancer subtypes and treating metastatic RCC patients (16, 17). Cancerous tissue RNA expression profiling has successfully identified aberrations at the molecular level that are clinically treatable (18). Studies have revealed that human renal tissues show distinct sexual dimorphism at molecular level. Where there is an elevated expression of X-linked tumor suppressor genes in women, men are more susceptible (2-fold) to RCC. The study of sex-dependent RCC advancement revealed prognostic markers that shape renal cancer and have clinical implications (19). Other transcriptomics applications include the assessment of tumor and circulating non-coding RNAs as prognostic biomarkers and understanding the effect of mutations in DNA, as some genetic aberrations are silenced at the RNA level (20).

The significance of omics sciences is further supported by mass spectrometry, which is the fundamental platform used for proteomics analysis (21). Proteomics research has revealed that soluble protein biomarkers and exosomes are an appealing diagnostic choice against RCC (22), because they are present in readily approachable medium (i.e., blood or urine) (18). Aquaporin-1 (AQP1), for example, functions as a selective transcellular transporter of water (23). AQP1 promotes the movement of membrane projections, which speeds up cell migration and is thus linked to angiogenesis and cancer advancement (24). It is found all over the human body and is upregulated in RCC. However, AQP1's expression is restricted to the kidney's proximal tubule. As a result, high urinary protein concentrations are probably because of RCC, making it a potential diagnostic choice against RCC (25). Moreover, the potential role of PLIN2, CAIX, and KIM1 as biomarkers for RCC has also been demonstrated by proteomics analysis (26).

Furthermore, while cancer was previously thought to be caused by aberrations in growth-regulating pathways, recent metabolomics research has reinforced the concept of adaptation of classical biochemical pathways to the tumor's benefit by the same genes that regulate tumor energetics and biosynthesis (27). Many clear renal cell carcinoma cells undergo the Warburg effect and reductive carboxylation (28, 29). This glutamine-dependent pathway involves the "backward flow" of the tricarboxylic acid (TCA) cycle, as well as an increase in an intrinsic oxidative stress buffer pathway, both of which aid tumor growth under unfavorable conditions (30). Such unexpected metabolic abnormalities in RCC have opened up new avenues for imaging as well as the development of new therapeutic targets (30). Table 1 reported the most frequently used omics science approaches in scientific research.

Table 1. Most frequently used omics science approaches in scientific research.

Omics Sciences	Biological Entity	Assay	Principle
Genomics	Nucleic acid (DNA or RNA)	Identify SNPs in the whole genome associated with clinical traits	Genotyping arrays/whole exome sequencing
Transcriptomics	Nucleic acid (RNA)	Quantify expression levels of cellular transcripts (mRNA)	Microarray/RNA sequencing
Proteomics	Protein	Characterize differential protein expression levels in cells/samples	MS-based and antibody-based approaches
Metabolomics	Low Molecular Weight Molecules (metabolites)	Differential metabolites expression	MS-based approaches

The Role of Omics Sciences in Kidney Cancer Diagnosis and Therapy

Multi-omics data integration strategies at different cellular function levels provide unprecedented opportunities to comprehend the underlying biology of kidney cancer (31). Multi-omics profiling is advantageous in providing a detailed apprehension of molecular alterations that lead to normal growth, cellular response, and disease. It also allows for identifying novel drug targets and biomarkers, promoting early diagnosis and allowing professionals to obtain fully detailed metabolic profiles from each patient, who is then treated with biological medicine. However, some of the challenges associated with the use of omics sciences in the diagnostic and therapeutic fields are linked to tumor heterogeneity, which makes identifying biomarkers that accurately reflect the characteristics of the entire tumor difficult. Furthermore, obtaining and analyzing sufficient tissue samples necessitates data integration from multi-omics platforms. Above all, there is a scarcity of prognostic molecular biomarkers in advanced kidney cancer (8).

Genetics of Kidney Cancer

One factor that can be linked to the increased risk of kidney cancer is genetic mutations or polymorphisms. Many Single Nucleotide Polymorphisms (SNP) play a role in developing kidney cancer. Genomic sequence analyses have identified SNPs in kidney cancer-related areas, such as 2p21, 2q22.3, 11q13.3, and 12p11.33 (32, 33). An individual's risk of developing kidney cancer can range greatly, from approximately 100% in the case of Hereditary Papillary Renal Cell to as low as 5% in the case of BAP1 (BRCA1-associated protein-1) tumor predisposition syndrome (34, 35). The genes related to kidney cancer are represented in **Table 2**. Despite the possibility of multiple founder mutations for each kidney cancer syndrome, most of these diseases are linked to numerous genetic variations. Most of these diseases are coding changes, with missense, nonsense, and insertion/deletion mutations occurring in various frequencies in the major genetic syndromes. Although there may be genotype-phenotype relationships in several disorders, further research is necessary to pinpoint the precise variant types linked to kidney cancer, because these illnesses are uncommon (36).

Table 2. List of genes linked to kidney cancer and the related syndromes.

Gene	OMIM of Gene	Gene Location	RCC Histologic Characteristic	Inheritance	OMIM of Pathology	Related Pathologies
VHL	608537	3p25.3	Renal cell carcinoma, somatic	AD	144700	• Erythrocytosis familial, 2 • Hemangioblastoma, cerebellar, somatic • Pheochromocytoma • von Hippel-Lindau syndrome
PTEN	601728	10q23.31	Renal cell carcinoma, clear cell (ccRCC)	AD	.	• Cowden syndrome 1 • Lhermitte-Duclos disease • Macrocephaly/autism syndrome • Prostate cancer, somatic • Glioma susceptibility 2 • Meningioma
MET	164860	7q31.2	Renal Cell carcinoma, papillary, 1, familial and somatic	AD	605074	• Arthrogryposis, distal, type 11 • Deafness, autosomal recessive 97 • Hepatocellular carcinoma, childhood type, somatic • Osteofibrous dysplasia
FH	136850	1q43	Leiomyomatosis and renal cell cancer	AD	150800	• Fumarase deficiency
FLCN	607273	17p11.2	Renal carcinoma, chromophobe, somatic	AD	144700	• Birt-Hogg-Dude syndrome • Colorectal cancer, somatic • Pneumothorax, primary spontaneous
BAP1	603089	3p21.1	Renal cell carcinoma (RCC)	Predisposition gene	.	• Kury-Isidor syndrome • Tumor predisposition syndrome 1 • Uveal melanoma
CHEK2	604373	22q12.1	Renal cell carcinoma (RCC)	.	.	• Li-Fraumeni syndrome 2 • Osteosarcoma, somatic • Breast cancer • Colorectal cancer • Prostate cancer, familial
CDC73	607393	1q31.2	Renal cell carcinoma (RCC)	.	.	• Hyperparathyroidism, familial primary • Hyperparathyroidism jaw tumor syndrome • Parathyroid adenoma with cystic changes • Parathyroid carcinoma

Segue

Continua

PBRM1	606083	3p21.1	Renal cell carcinoma, clear cell (ccRCC)	.	144700	
OGG1	601982	3p25.3	Renal cell carcinoma, clear cell, somatic	.	144700	.
RNF139	603046	8q24.13	Renal cell carcinoma (RCC)	.	144700	.
HNF1A	142410	12q24.31	Renal cell carcinoma (RCC)	.	144700	• Diabetes mellitus, insulin-dependent, 20 • Hepatic adenoma, somatic • MODY, type III
HNF1B	189907	17q12	Renal cell carcinoma (RCC)	.	144700	• Renal cysts and diabetes syndrome • Type 2 diabetes mellitus

Genomics of Kidney Cancer

Genomic research has revealed various genetic and epigenetic changes in kidney cancer. VHL (regulating angiogenesis), PBRM1, SETD2, and BAP1 (regulating epigenetic processes such as chromatin and histone modification) are the most mutated genes in clear cell RCC (37). Many of these genes' prognostic significance has been the subject of active research. Since molecular classification can predict response to therapy, it paves the way for a more personalized approach to kidney cancer treatment. Several tumor-specific factors, such as VHL mutations and hypoxia-inducible factor levels, have been investigated as potential biomarkers for VEGF-targeted therapy. None of these are currently in clinical use and require additional prospective validation (38).

Pharmacogenomics

Pharmacogenomics helps understand the impact of genetic variants on drug toxicity and effectiveness (39). In the past, cytokine therapies (such as interleukin-2 and interleukin-alpha) were the only accessible therapy choice, but they have had poor efficacy and severe toxicity for the past few years (40, 41). For the treatment of metastatic Renal Cell Carcinoma (mRCC), various targeted treatments have been developed during the past ten years. Among these treatments, the most important is the oral multi-targeted tyrosine kinase inhibitors (TKIs) with antiproliferative efficacy. Sorafenib, sunitinib, and pazopanib were the very first batch of TKIs to receive approval for the treatment of mRCC. In 2012, second-generation TKIs began to appear to treat mRCCs, including axitinib, tivozanib, cediranib, and cabozantinib (42). Different drugs used as the therapeutics for the treatment of mRCC are mentioned in Table 3.

Table 3. Drugs used for the treatment of mRCC along with their targets and mechanism of action.

Drug	Treatment Line	Target	Mechanism of action
Pembrolizumab	First	PD-1	Monoclonal antibody
Axitinib	Second	VEGFR1, VEGFR2,VEGFR3	Multiple receptor tyrosine kinase inhibitor
Avelumab	Second	PD-L1	Monoclonal antibody
Nivolumab	First	PD-1	Monoclonal antibody
Ipilimumab	First	CTLA4	Monoclonal antibody
Cabozantinib	Second	VEGFR, AXL, MET	Multiple receptor tyrosine kinase inhibitor
Lenvatinib	First	VEGFR1, VEGFR2, VEGFR3, FGFR1, FGFR2, FGFR3, FGFR4, PDGFR, KIT, RET	Multiple receptor tyrosine kinase inhibitor
Everolimus	Second and third	mTOR	Rapalog
Pazopanib	First	VEGFR1, VEGFR2, VEGFR3, PDGFR, KIT	Multiple receptor tyrosine kinase inhibitor
Bevacizumab	First	VEGF	Monoclonal antibody
Temsirolimus	First	mTOR	Rapalog
Sunitinib	First	VEGFR, PDGFR	Multiple receptor tyrosine kinase inhibitor
Sorafenib	First	VEGFR, PDGFR, KIT, RET	Multiple receptor tyrosine kinase inhibitor

cfDNA

Among the non-invasive methods used for cancer diagnosis and detection, one is cell-free DNA (cfDNA) detection. cfDNA is present in plasma as an extracellular DNA and is based on the cancer cell components released into the systemic circulation, making the diagnosis of cancer possible through the "liquid samples." Pregnancy is one scenario in which the amount of cfDNA was observed to increase (43). The other conditions include systemic inflammatory response syndrome (44), malignancies (45), and urinary tract infections (46). Recently, the detection of renal cell carcinomas has been connected to cfDNA, which has previously been successful in the diagnosis of many malignancies (47).

Moreover, cfDNA levels are linked to the tumor's grade and stage and to RCC metastatic load (48). Preoperative cfDNA levels were higher in patients undergoing nephrectomy for metastatic and organ-confined diseases. Tumor grade and nodal status of patients with organ-confined illness are not linked to cfDNA (49, 50).

The function of cfDNA in detecting and treating kidney cancer was analyzed in different studies. It has been reported that the cfDNA level was lower in patients that have undergone the removal of malignant tumors (51). Although all patients' cfDNA levels dropped after surgery, they rose before clinical recurrence, but in patients without any recurrence they remained low. Patients with clinically restricted disorders who experienced recurrence had a higher preoperative concentration of cfDNA than those who did not. In predicting recurrence, the post-operative concentration of cfDNA performed better than clinical and pathological characteristics (50).

Metabolomics of Kidney Cancer

The ability of metabolomics to identify biomarkers in both biofluids and tissues makes it an effective method for this goal (52). Metabolomics methods that perform mass spectrometry (MS) in combination with nuclear magnetic resonance have allowed researchers to systematically quantify hundreds of metabolites from a single biological sample (NMR) (53, 54).

Proteomics and biomarkers

A specialized screening technique is usually needed for malignancies of reduced incidence, like RCC. One method would be to use a urine biomarker to expand the sample size of the high-risk screening population (55-57). Using biomarkers found in blood and urine makes early detection of RCC possible. When it involves evaluation, biomarkers are only useful if they have been subjected to analytical and clinical validation. Detecting biomarkers can improve RCC prognosis and treatment (57-59). Various RCC types are differentiated based on distinct biomarkers, among which promising ones are exosomes, soluble proteins, microRNA, and circulating tumor DNA (22, 60). Urinary proteome studies offer a promising new approach for detecting RCC at an early stage because they are accessible, quantifiable, and located near the diseased organ. Furthermore, urine protein contents are usually low without parenchymal renal dysfunction (61).

Proteomics can identify biomarkers and therapeutic targets in health and disease systems biology. Precision medicine and proteomics help precision oncology analyze complicated carcinogenic pathways and targeted therapies, find novel biomarkers for screening and detection, and evaluate therapy effectiveness and toxic effects (62). Some of the major proteomics biomarkers responsible for renal cell carcinoma are mentioned in table 4.

Lipid omics and biomarkers

A recent study demonstrated that high SETD8 expression is highly linked to lipid accumulation, higher tumor progression, and a worse prognosis in individuals diagnosed with renal cell carcinoma (63). Increased 2-hydroxyglutarate concentrations have been associated with epigenetic changes that cause neoplasia (64). Clear-cell renal cell carcinoma (ccRCC), the most prevalent kind of RCC, is known for lipid metabolism abnormalities, since it relies on extracellular fatty acids (FA) for nutrition, especially during metabolic stress (65). The buildup of cholesterol, other lipids, and esters in intracellular lipid droplets (LDs) is a defining characteristic of RCC. These lipids are responsible for lipid intake and storage, as well as for homeostasis maintenance, energy generation, and membrane biosynthesis. FA-oxidation

Table 4. List of Proteomics biomarkers linked to kidney cancer.

Biomarker	Protein family	Protein class	Function
PLIN2	Perilipin family	Metabolic proteins, Plasma proteins	Role in renal cancer
CAIX	Large family of zinc metalloenzymes	Enzymes, Metabolic proteins, Transporters	Expressed in all clear-cell renal cell carcinoma (ccRCC)
KIM-1	Immunoglobulin	CD markers	Renal cancer
RKIP	Phosphatidylethanolamine-binding family of proteins	Plasma proteins	Marker in renal cell carcinoma (RCC)
VEGF	Plasma proteins, RAS pathway-related proteins	PDGF/VEGF growth factor family	Cancer enhanced (renal cancer)

enzyme activity decreases with tumor progression, size, grade, and survival (66, 67). PLIN2 and HILPDA are the two overexpressed proteins of the LD in ccRCC, which control lipid storage and increase the proportion of lipids with polyunsaturated fatty-acyl side chains. Also, both isoforms of AnxA3, which control adipocyte development negatively, are differentially expressed in RCC (68, 69). Signaling pathways are found to be associated with cholesterol metabolism (i.e., AKT, RAS, and SRC), which can result in cancer development (70, 71). LAL and VLDL-R have higher expression in RCC, which leads to a reduced survival rate (72).

Microbiomics

Much scientific research and description have been devoted to understanding RCC epidemiology (73). On the other hand, the importance of the contribution of previous UTIs to the occurrence of RCC is only partially understood. It has been reported that urinary tract infections are a modifiable factor that promotes renal cell carcinoma growth (74). Although the relationship between microbiota and RCC development has not been investigated, the recent identification of a unique urinary tract microbiome raises the idea that there is a greater degree of direct contact between the microbiota and the kidney than previously recognized. Now, having access to the tools to isolate and identify individual bacterial species, researchers can do more detailed studies of the urine microbiome of renal cell carcinoma (75). In advanced cancer patients with few treatment options, gut microbiota can boost the effects of immunotherapy. However, a few questions still need to be solved before microbiome therapy can be implemented into ordinary clinical practice (76). Some of the major microbiome biomarkers associated with RCC are listed in table 5.

Future directions and conclusions

Our perspective of kidney cancer has been fundamentally altered by the omics sciences, which have opened up novel possibilities for diagnosing and treating the disease. Data from genomics, metabolomics, proteomics, and transcriptomics have been integrated, which has led to the discovery of possible therapeutic targets and biomarkers. Omics will help to further clarify the metabolic pathways, including MAPK and PI3K/AKT pathways. Different novel targets and biomarkers can be identified for clinical applications by studying the oncometabolite and its associations with different signaling pathways. Multiple lipids identifications as negative association with RCC, including phosphatidylcholines and plasmalogens, can act as promising biomarkers.

Diagnostic and prognostic accuracy and reliability have been increased using omics data. Treatment strategies can be developed based on omics, with the help of personalized

Table 5. Microbiome biomarkers associated with RCC.

Biomarker	Characteristics	Family	Microbiome	Expression Status	Role in carcinogenesis
Akkermansia muciniphila	Oval-shaped, mucin-degrading bacterium	Akkermansiaceae	Intestinal Mucosa (symbiont)	High	Triggers host metabolic response and promotes inflammatory cytokine production and release.
Blautia obeum	Usually spherical or oval probiotic	Lachnospiraceae	Feces and GIT	High	Produces short chain fatty acids associated with both pro- and anti-tumor effects, depending on the context.
Ruminococcus bromii	Cocci-shaped, it degrades and converts complex polysaccharides into a variety of nutrients for its hosts	Oscillospiraceae	Gut	High	Produces host DNA-damaging genotoxic metabolites, modulates host's immunity and promotes inflammatory cytokines release.
Streptococcus bovis	Cocci-shaped human pathogen (UTIs)	Streptococcaceae	GIT	High	Produces inflammation and proliferation, promoting virulence factors.
Bacteroids fragilis	Pleomorphic to rod-shaped, related to many clinical infections	Bacteroidaceae	Colon	High	Damages host DNA by producing genotoxins and activates oncogenic pathways.
Prevotella intermedia	Rod-shaped, putative periodontal pathogen	Prevotellaceae	Mucosal sites	Low	Promotes tumor growth, metastasis, and inflammation.
Streptococcus lutetiensis	Cocci-shaped, causing sepsis in adults	Streptococcaceae	GIT	High	Favors cellular proliferation, migration, and invasion.

medicine. Efficient, targeted therapy can produce fewer toxic effects on normal cells than chemotherapy. It can be a successful approach for an early kidney cancer diagnosis; further research can lead to more effective treatments.

The heterogeneity of tumor microenvironment and therapy of specific types of cancer can be better comprehensible with the help of various omics technologies. The diagnostic test has yet to reach a point where it can be used in a clinical area. More research is needed to validate biomarkers, determine their therapeutic potential, and integrate the proper protocols. Possible biomarker tests may be developed using miniaturized assays and multiplexing technology.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review *Clin Ter 2023; 174 Suppl. 2 (6):55-67 doi: 10.7417/CT.2023.2472*

Omics sciences and precision medicine in colon cancer

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Abstract

Colon cancer presents a complex pathophysiological landscape, which poses a significant challenge to the precise prediction of patient prognosis and treatment response. However, the emergence of omics sciences such as genomics, transcriptomics, proteomics, and metabolomics has provided powerful tools to identify molecular alterations and pathways involved in colon cancer development and progression. To address the lack of literature exploring the intersection of omics sciences, precision medicine, and colon cancer, we conducted a comprehensive search in ScienceDirect and PubMed databases. We included systematic reviews, reviews, case studies, clinical studies, and randomized controlled trials that were published between 2015-2023. To refine our search, we excluded abstracts and non-English studies. This review provides a comprehensive summary of the current understanding of the latest developments in precision medicine and omics sciences in the context of colon cancer. Studies have identified molecular subtypes of colon cancer based on genomic and transcriptomic profiles, which have implications for prognosis and treatment selection. Furthermore, precision medicine (which involves tailoring treatments, based on the unique molecular characteristics of each patient's tumor) has shown promise in improving outcomes for colon cancer patients. Omics sciences and precision medicine hold great promise for identifying new therapeutic targets and developing more effective treatments for colon cancer. Although not strictly designed as a systematic review, this review provides a readily accessible and up-to-date summary of the latest developments in the field, highlighting the challenges and opportunities for future research. ***Clin Ter 2023; 174 Suppl. 2 (6):55-67 doi: 10.7417/CT.2023.2472***

Key words: *Tumor genomics, pharmacogenomics, proteomic biomarkers, metabolomic, germline mutations.*

Background

Colon cancer is a significant contributor to morbidity and mortality across the globe (1). It is a multifaceted disease, caused by the aggregation of numerous genetic and epigenetic changes that arise at the cellular level in the colon. The use of omics disciplines has significantly enhanced our comprehension of the molecular pathways underlying colon cancer (2). These advancements have facilitated the development of more personalized and precise approaches to managing the disease. The various omics sciences—including genomics, transcriptomics, proteomics, and metabolomics—have revolutionized our understanding of cancer biology (3). Although significant evidence has emerged in favor of single omics approach for the detection of genetic and molecular mutations, their capacity to establish causal relationships between molecular signatures and phenotypic expressions of cancer hallmarks is restricted. Compared to single omics, the multi-omics approach can uncover the underlying complexities, such as metastasis and angiogenesis (4). The analysis of vast molecular data using these technologies from cancer cells offers a comprehensive view of genetic, epigenetic, and metabolic changes that drive tumor development and progression.

Gene expression profiling has revealed differentially expressed genes associated with tumor initiation, progression, and metastasis (5). Additionally, transcriptomic analysis has identified different molecular subtypes of colon cancer, each exhibiting unique gene expression profiles and clinical features. Proteomics and metabolomics have also emerged as valuable tools for identifying novel biomarkers and therapeutic targets in colon cancer. Proteomic analysis identifies

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differentially expressed or post-translationally modified proteins in colon cancer, providing insights into the molecular pathways that are dysregulated in the disease (6). Precision medicine, which involves individualizing therapy based on a patient's molecular profile, has emerged as a promising approach to colon cancer treatment (7). Omics-based profiling of colon cancer enables the identification of specific molecular changes that drive tumor growth, thus allowing the selection of targeted therapies likely to be effective in each patient. Furthermore, precision medicine allows to identify patients at high risk of disease recurrence, enabling the development of personalized surveillance strategies and the optimization of adjuvant therapy (8).

Aim of the review

Currently, a dearth of literature exists concerning reviews that comprehensively explore the present understanding of omics sciences and precision medicine in colon cancer. The primary objective of this manuscript is to deliberate on the current body of evidence regarding the latest developments in omics science and precision medicine in the context of colon cancer.

Methodology

To identify the appropriate studies, we comprehensively searched ScienceDirect and PubMed databases using individual terms and Boolean operators ANDs and ORs. The search terms used included "genetic mutations," "germline genetic mutations," "genetic tests," "hereditary," "somatic genetic mutations," "genetic rearrangements," "proteomics biomarkers," "blood proteomics biomarkers," "clinical diagnosis," "therapy," "pharmacogenetics," "metabolomics," "microbiomics," and "colon cancer." We limited our inclusion criteria to specific types of articles, including meta-analyses, multicenter studies, reviews, systematic reviews, observational studies, case-control studies, longitudinal/prospective studies, retrospective studies, and randomized controlled trials. We refined our search by limiting the publication date between 2015-2023 and excluding all non-English studies. Furthermore, texts available only in abstract form were excluded.

Tumor natural history and clinical history

Colon cancer originates from the epithelial cells of the colon. The natural history of colon cancer involves a sequence of genetic, molecular, and cellular events that ultimately lead to disease development and progression (9). The initial step in the natural history of colon cancer involves the transformation of a normal colonic epithelial cell into a neoplastic cell, which is manifested by mutations in specific genes, like the adenomatous polyposis coli gene. Once these neoplastic cells have formed, they begin to proliferate and accumulate genetic alterations that enable them to evade normal cellular control mechanisms—such as apoptosis and immune surveillance. These genetic alterations comprise mutations affecting oncogenes and tumor suppressor genes, alongside alterations in DNA methylation patterns and chromatin conformation (10). Over time, these neoplastic cells may form a polyp. Furthermore, the spread of colon cancer is facilitated by the development of blood and lymphatic vessels within the tumor, which provides a pathway for cancer cells to enter the circulation and establish secondary tumors. The clinical history of colon cancer can vary depending on the stage and location of the tumor. Early-stage colon cancer may not cause any symptoms and the disease may be detected incidentally during routine screening tests, such as colonoscopy. As the tumor grows and invades the surrounding tissue, it can cause symptoms like rectal bleeding, abdominal pain, and weight loss. These symptoms may be nonspecific and can also be caused by other gastrointestinal conditions, such as inflammatory bowel disease and diverticulitis (1).

Genetics of the tumor

The development of colon cancer involves multiple mutations at the genetic level, DNA methylation alterations, and changes in gene expression (Table 1). These genetic alterations can impact certain cellular processes that regulate cell proliferation and apoptosis, leading to the uncontrolled growth of cancer cells (11). The most frequent genetic aberration in colon cancer is chromosomal instability (CIN), which is characterized by extensive chromosomal aberrations and loss of heterozygosity. This subtype is frequently marked by mutations in key genes, including APC, KRAS, TP53, PI3KCA, and SMAD4. According to a study by Kandioler et al., TP53 mutation was observed in 33% of stage 3 colon cancer patients (12). Interestingly, the study also revealed that TP53 wild-type subjects had a significantly better survival rate than those with TP53 mutations (81% vs. 62%) (12). The most frequently observed cytogenetic abnormality in colon cancer is the loss of heterozygosity on chromosome 18q. Li et al. conducted a study where they investigated copy number variations (CNVs) of plasma cell-free DNA (cfDNA) in cancer (n=80), polyps (n=20), and healthy controls (n=35) using sequencing-based copy number analysis (13). Their results revealed that there were frequent CNVs in various chromosomal regions, such as amplifications on 1q, 8q, and 5q, as well as deletions on 1p, 4q, 8p, 17p, 18q, and 22q (13). These findings were consistent with a previous study conducted by Mampaey et al., who also reported frequent gains in chromosomes 1q, 7, 8q, 13, and 20, as well as losses for 1p, 4, 8p, 14, 15, 17p, 18, 21, and 22 (14).

Colon cancer can exhibit genetic alterations such as the CpG Island Methylator Phenotype (CIMP), where abnormal methylation of CpG islands results in the silencing of tumor suppressor genes (15). CIMP-high tumors are a distinct molecular subgroup that exhibit specific genetic characteristics, such as wild-type P53, microsatellite instability (MSI), and mutated BRAF. A phase 2 study, conducted by Watanabe et al., investigated the clinical significance of RAS/BRAF mutations in circulating cell-free DNA (ccfDNA) (16). The study enrolled 54 patients, 17 of which showed RAS/BRAF mutations at the end of the treatment protocol, while 10

patients had RAS/BRAF mutations in their plasma ccfDNA at the baseline of the study (16). Guda and colleagues recently employed whole exome sequencing and targeted sequencing to uncover somatic mutations in 103 patients. Their study revealed 20 previously unidentified genes in African American CRC patients. Notably, two genes, ephrin type A receptor 6 (EPHA6) and folliculin (FLCN), exclusively mutated in African American CRCs, were identified as potential driver genes based on genetic and biological criteria (17). Similarly, Thai patients with stage 2 and 3 colon cancer exhibited a mutation frequency of 47.2%, 1.9%, 1.9%, 12%, and 14.8% in KRAS, NRAS, BRAF, PIK3CA, and FBXW7, respectively (18).

Genetic testing

The integration of genetic testing in healthcare has been guided by accumulating evidence and established guidelines (19). Initially, patients were evaluated based on observable characteristics or "phenotypes," such as family history, individual risk factors, and tumor characteristics. The use of next-generation sequencing resulted in a shift towards multigene panel testing, which enables the identification of germline mutations that might not have been discovered based on observable characteristics and family history (20). This method has several advantages, including improving the identification of germline mutations for syndromes with genetic heterogeneity and overlapping characteristics (21). Limiting germline testing based on observable characteristics may cause the omission of certain cancer susceptibility genes.

Another diagnostic approach to emerge in colon cancer is stool DNA screening (22). A recent study evaluated the effectiveness of the novel stool DNA test of methylated SDC2 for colon cancer detection, which revealed high sensitivity for detecting colon cancer and advanced adenomas

Table 1. Summary of some of the genes involved in colon cancer, their associated pathologies and OMIM numbers, the inheritance patterns, and the effects of the mutations.

Gene	OMIM	Pathology	OMIM	Inheritance	Pathways/Functions	Effects of mutations
APC	611731	Familial adenomatous polyposis	175100	Autosomal dominant	Wnt signaling pathway; Regulation of cell growth and differentiation	Loss of function mutations lead to the formation of multiple colorectal polyps, which have a high probability of becoming malignant tumors.
MLH1	120436	Lynch syndrome 2	609310	Autosomal dominant	DNA mismatch repair pathway	Mutations increase the risk of colorectal, endometrial, and other cancers by causing genomic instability and accumulation of mutations.
MSH2	609309	Lynch syndrome	120435	Autosomal dominant	DNA mismatch repair pathway	Mutations increase the risk of colorectal, endometrial, and other cancers by causing genomic instability and accumulation of mutations.
MSH6	600678	Lynch syndrome 5	614350	Autosomal dominant	DNA mismatch repair pathway	Mutations increase the risk of colorectal, endometrial, and other cancers by causing genomic instability and accumulation of mutations.
PMS2	600259	Lynch syndrome 4	614337	Autosomal dominant	DNA mismatch repair pathway	Mutations increase the risk of colorectal, endometrial, and other cancers by causing genomic instability and accumulation of mutations.
KRAS	190070	Colorectal cancer	114500	Somatic	MAPK signaling pathway	Gain-of-function mutations activate KRAS, leading to uncontrolled cell proliferation and tumor growth.
TP53	191170	Li-Fraumeni syndrome	151623	Autosomal dominant	Regulation of cell cycle and apoptosis	Mutations impair the tumor suppressor function of TP53, leading to an increased risk of cancer.
BRAF	164757	Colorectal cancer	114500	Somatic	MAPK signaling Pathway	Oncogene, stimulates cell growth and division.
SMAD4	600993	Juvenile Polyposis Syndrome	175050	Autosomal Dominant	TGF-beta signaling Pathway	Tumor suppressor, regulates cell division.
PIK3CA	171834	Colorectal cancer	114500	Somatic mutations	PI3K/AKT signaling pathway	Oncogene, promotes cell growth and survival.

(23). Colon cancer is linked with dysregulated expression of microRNAs (miRNAs), and the expression patterns of these small non-coding RNAs have been linked to the detection and prognosis of colon cancer. A recent review demonstrated the effectiveness of using circulating serum miRNA and fecal miRNA expression as non-invasive biomarkers for early detection of colon cancer (24).

Predisposition

The pathogenesis of colon cancer is multifactorial, involving both genetic predisposition and various environmental factors. Genome-wide linkage analyses have revealed significant correlations between susceptibility loci located on chromosome 8p23 and colon cancer (25). Genetic susceptibility to colon cancer involves modifications in gene expression and DNA methylation, with specific genes being identified as markers for different subtypes of cancer. Additionally, the inactivation of microRNA through DNA methylation can also contribute to the development of colon cancer (26). Furthermore, the epigenetic inactivation of genes responsible for regulating the cell cycle, angiogenesis, repairing DNA, and promoting cellular differentiation are also contributors to colon cancer (27). Although the role of innate immunity genes in the progression of colon cancer is unclear, a weaker immune response is usually manifested in different cancers. Toll-like receptors (TLRs) play a vital role in identifying pathogen-associated molecular patterns, which trigger the innate immune response. Upon activation, TLRs activate NF-κB, which further initiates the transcription of various pro-inflammatory cytokines, and human beta-defensins (hBDs) (28). hBDs are antimicrobial peptides that aid innate immune defense, with hBD-1 being produced by various epithelial tissues, and their expression can be induced. Additionally, upon encountering microorganisms or cytokine stimulation, hBD-2 is highly expressed in normal epithelial cells. Semlali et al. conducted a study that explored the genetic variations and expression of hBDs (hBD-1, hBD-2, hBD-3, and hBD-4) and their potential association with colon cancer. Their findings revealed a significant association between hBDs and the deregulation of innate immunity [29]. These results suggest that hBDs play a critical role in maintaining innate immunity and that their disruption may contribute to the pathogenesis of colon cancer (29).

Correlated syndromes

In addition to sporadic colon cancer, also several colon cancer syndromes have been identified. These syndromes are characterized by a genetic predisposition to colon cancer, often with a specific pattern of inheritance. One of the most well-known colon cancer syndromes is hereditary nonpolyposis colorectal cancer (HNPCC), also known as Lynch syndrome. HNPCC is an autosomal dominant disorder caused by mutations in one of several DNA mismatch repair genes (30). Individuals with HNPCC have a significantly increased risk of developing colon cancer, as well as other cancers such as endometrial, ovarian, and gastric cancer. The identification of HNPCC in families is important, because it can guide screening and surveillance protocols, such as colonoscopies starting at an earlier age or more frequent intervals. Another colon cancer syndrome is familial adenomatous polyposis (FAP), which is also an autosomal dominant disorder. FAP is characterized by the development of hundreds to thousands of adenomatous polyps in the colon, which can progress to cancer if left untreated (31). FAP is caused by mutations in the APC gene, which regulates cell proliferation and differentiation. Individuals with FAP require regular colonoscopies and often undergo prophylactic surgery to remove the colon, to prevent the development of colon cancer. In addition to HNPCC and FAP, there are several other colon cancer syndromes, such as MUTYH-associated polyposis (32), Peutz-Jeghers syndrome (33), and juvenile polyposis syndrome (34). These syndromes are less common than HNPCC and FAP, but still have important implications for patient management and surveillance. The identification of these syndromes can guide appropriate screening and surveillance protocols and help to prevent the development of colon cancer in at-risk individuals.

Genomics of the tumor

Recent advancements in genomic technologies have provided a comprehensive understanding of the genetic changes underlying the development and progression of colon cancer (Table 2). Among the most frequently observed genomic alterations in colon cancer is the mutation of the adenomatous polyposis coli (APC) gene (35). The APC gene is a tumor suppressor that plays a crucial role in regulating the Wnt signaling pathway, which is responsible for controlling cell proliferation and differentiation. APC mutations are identified in up to 80% of colon cancer cases, and they are thought to be an early event in the pathogenesis of the disease (36, 37). In addition to APC mutations, colon cancer is characterized by the accumulation of additional genomic alterations, including mutations in oncogenes such as KRAS, NRAS, and BRAF (38), as well as in other tumor suppressor genes such as TP53, SMAD4, and PTEN (39). Recent studies have also highlighted the importance of epigenetic alterations in colon cancer (40, 41). Epigenetic alterations including DNA methylation and histone modifications affect gene expression without altering the underlying DNA sequence. In colon cancer, aberrant DNA methylation patterns have been found including the MLH1, MGMT, and CDKN2A genes (42). These genetic modifications can result in the loss of function of tumor suppressor genes and the gain of function of oncogenes which consequently contribute to tumor progression (43).

Rearrangements

Multiple genetic alterations, including gene rearrangements, have emerged as a hallmark of colon cancer. Gene rearrangements occur when two or more regions of DNA from different chromosomes are broken and then rejoined in a new order. These rearrangements can lead to changes in gene expression and protein function, ultimately contributing to the development and progression of colon cancer (54).

Table 2. The potential genomics biomarkers in colon cancer.

Author, Year	Biomarker	Methodology	Change	Results	Conclusion
Song et al. (2018) (44)	CBX8, CD96	Gene and isoform expression datasets from The Cancer Genome Atlas	Downregulated	2301 genes and 4241 isoforms were differentially expressed	CBX8 and CD96 are viable prognostic biomarkers
Zhu and Dong (2018) (45)	TUSC3	Oncomine and COEXPEDIA databases	Upregulated	TUSC3 mRNA expression was overexpressed in CRC tissues compared to the control ones	TUSC3 is a potential therapeutic target in CRC
Luo et al. (2020) (46)	MTMR7, GSTM5, GPX2, PDE6B, CDS1, SGPP2, GSTM2, ALDOB, CPT1C, PDE1B, AGMAT, FTCD, HDC, DGKB, ACADL, MAT1A, PLCG2	The Cancer Genome Atlas (TCGA), Genotype-Tissue Expression (GTEx) database, and Gene Expression Omnibus (GEO)	43 mRNAs were upregulated, whereas 104 mRNAs were downregulated	A seventeen-gene metabolic signature emerged as prognostic biomarker. The high-risk patient group had a poor prognosis when compared to low risk (HR: 1.174, P < 0.001)	Seventeen-gene metabolic signature is a potential prognostic biomarker for colon cancer
Cheng et al. (2022) (47)	MTUS1	Tumor tissues were analyzed by qPCR for MTUS1 expression	Downregulated	MTUS1 exhibited lower levels in tumor tissues as compared to normal tissues	MTUS1 can serve as a prognostic and diagnostic biomarker for colon cancer
Chen et al. (2019) (48)	SEPT9, SDC2, NDRG4	Stool samples from cancerous and non-cancerous patients	Upregulated	DNA methylation of SEPT9, NDRG4, and SDC2 showed efficacy for diagnosis of colon cancer, but not BMP3	Potential screening biomarkers for colon cancer
Zhang et al. (2022) (49)	SDC2, TFPI2	Stool samples	Upregulated	Methylation levels of SDC2 and TFPI2 were higher in tumor samples as compared to normal samples	In CRC, SDC2 and TFPI2 were hypermethylated
Moradi et al. (2020) (50)	SOX21	The MethyLight method was utilized to determine methylation levels in the stool	Upregulated	The methylation rates of SOX21 were significantly higher in tumor tissues compared to normal tissues (P < 0.0001)	SOX21 gene promoter methylation is a potential diagnostic biomarker for colon cancer
Alizadeh-Sedigh et al. (2022) (51)	PIK3CA, KRAS, BRAF	PCR-direct sequencing for PIK3CA mutations	-	PIK3CA exon 9 (47.1%) in cancerous tissues	PIK3CA, KRAS, BRAF, and APC hotspot mutations have diagnostic potential in colon cancer
Ghatak et al. (2022) (52)	BDNF, PTGS2, GSK3B and CTNNB1, HPGD	Tissue samples	Upregulated	BDNF, PTGS2, GSK3B, and CTNNB1 were upregulated, whereas HPGD was significantly downregulated	Prognostic and diagnostic biomarkers
Ahluwalia et al. (2019) (53)	YWHAB, MCM4, and FBXO46	The Cancer Genome Atlas, COAD, and READ datasets	Upregulated	DPP7/2, YWHAB, MCM4, and FBXO46 were found to be significant predictors of poor prognosis in CRC patients (HR: 3.42, 95%, p < 0.001)	Potential prognostic biomarkers

One well-studied gene rearrangement in colon cancer involves the fusion of the EML4 (echinoderm microtubule-associated protein-like 4) gene and the ALK (anaplastic lymphoma kinase) gene. This rearrangement results in the constitutive activation of the ALK tyrosine kinase, which promotes cell proliferation and survival. The EML4-ALK fusion has been identified in a small subset of CRC patients, and its presence is associated with poor prognosis (55, 56). Another common gene rearrangement in CRC involves the fusion of the BRAF (v-raf murine sarcoma viral oncogene homolog B) gene and the KIAA1549 gene (57). This rearrangement results in the overexpression of the BRAF protein, which is involved in the RAS/RAF/MEK/ERK signaling pathways. The BRAF-KIAA1549 fusion has been identified in a significant proportion of CRC patients with microsatellite instability (MSI), which is a hallmark of defective DNA mismatch repair. The presence of this fusion is associated with a better prognosis in MSI CRC patients (58).

In addition to these specific gene rearrangements, chromosomal instability (CIN) is a hallmark of CRC, and it can result in a variety of gene copy number alterations, including deletions, amplifications, and translocations. For example, the loss of the tumor suppressor genes APC (adenomatous polyposis coli) and TP53 (tumor protein p53) is a common event in CRC that can result from chromosomal deletions. On the other hand, amplifications of the oncogene MYC (MYC proto-oncogene, bHLH transcription factor) have also been observed in CRC, and these amplifications can lead to increased MYC expression and tumor cell proliferation (59). A study by Créancier et al. reported 2 colon cancer cases (out of 408) with NTRK1 chromosomal rearrangements, with one manifested as TPM3–NTRK1 fusion and other as TPR–NTRK1 fusion (60).

Fusion genes

One important mechanism of genetic alteration in colon cancer is the formation of fusion genes. Fusion genes are created when two previously separate genes become linked together through a chromosomal rearrangement, such as a translocation or inversion (61). This results in a new gene that encodes a fusion protein, which can have altered or novel functional properties compared to the original proteins. Several fusion genes have been identified in colon cancer, with the most common involving the genes encoding the transcription factor ETS-related gene (ERG) and the receptor tyrosine kinase Ret (62, 63). The ERG gene is normally involved in regulating gene expression during development and differentiation, while Ret is involved in cell growth and survival signaling. When these two genes are fused together, the resulting ERG-Ret fusion protein has constitutive tyrosine kinase activity, leading to uncontrolled cell growth and proliferation. Other fusion genes identified in colon cancer include those involving the BRAF gene, which is frequently mutated in colon cancer, and the neurotrophic receptor tyrosine kinase 2 (NTRK2) gene. The BRAF fusion gene results in the activation of the MAPK signaling pathway, which promotes cell growth and survival (64). The NTRK2 fusion gene produces a fusion protein with constitutive kinase activity, leading to increased cell proliferation and survival (65). The detection of fusion genes in colon cancer has important implications for diagnosis, prognosis, and treatment. Fusion genes can serve as biomarkers for the identification of colon cancer subtypes with different clinical outcomes and responses to therapy.

Somatic mutations

Somatic mutations, which occur in non-germline cells, are a critical driver of colon cancer pathogenesis. These mutations affect various genes that participate in diverse cellular pathways, including those regulating cell cycle progression, DNA damage response, and cellular signaling. The adenomatous polyposis coli (APC) gene, which is a tumor suppressor gene that controls cell proliferation and differentiation, is one of the most commonly mutated genes in colon cancer. APC mutations are present in 80% of sporadic colon cancer cases, and loss of APC function results in the accumulation of κcatenin, a transcriptional co-activator that stimulates cell proliferation and survival (35). Colon cancer is characterized by the presence of mutations in several key genes, including APC, KRAS, and TP53. KRAS mutations are particularly prevalent, occurring in around 40% of cases (66). These mutations activate the RAS/MAPK-signaling pathway, which plays a critical role in promoting cell proliferation and survival. In addition, TP53 mutations are also common, occurring in approximately 50% of cases (67). Loss of TP53 function results in the loss of its tumor suppressor activity, which normally regulates cell cycle arrest and apoptosis in response to DNA damage. Furthermore, colon cancer involves somatic mutations in various genes that regulate critical cellular processes, such as DNA repair. For example, the MutS homolog 2 (MSH2) and MutL homolog 1 (MLH1) genes, which play a crucial role in mismatch repair, are often affected by somatic mutations in colon cancer (68). Such mutations can lead to a genetic condition called microsatellite instability, which causes the accumulation of errors in repetitive DNA sequences.

With the advent of modern sequencing technology, it has become possible to identify additional somatic mutations that contribute to the development of colon cancer. For instance, mutations have been detected in genes that regulate chromatin remodeling, such as the SWItch/Sucrose Non-Fermentable (SWI/SNF) complex and the Polycomb repressive complex 2 (PRC2) (69). Rosic et al. conducted a genetic analysis on a cohort of 80 colon cancer patients, which uncovered a novel somatic variation in the form of an imbalance in alleles of single nucleotide variants (SNVs) (70).

Circulating tumor DNA

Circulating tumor DNA (ctDNA) has emerged as a promising biomarker for the diagnosis, prognosis, and treatment of colon cancer, which is a prevalent type of cancer worldwide. ctDNA comprises DNA fragments that are released by tumor cells into the bloodstream and can provide valuable information about the genetic characteristics of the tumor. Studies have revealed that ctDNA is present in nearly all patients with advanced colon cancer, and its levels are associated with the stage and burden of the disease (71, 72).

ctDNA can also be detected in patients with early-stage colon cancer, albeit at lower levels. The detection of ctDNA in the peripheral blood of colon cancer patients is a sensitive and specific method for detecting residual disease after surgery or monitoring the response to treatment. ctDNA analysis offers significant benefits beyond its diagnostic and prognostic value. For example, ctDNA can detect mutations in genes such as KRAS and TP53 that are commonly mutated in colon cancer. ctDNA analysis can also provide information about other genetic alterations—such as microsatellite instability (MSI) or BRAF mutations—that are associated with poor prognosis in colon cancer (73).

Pharmacogenomics

Pharmacogenomics is a discipline that investigates the influence of an individual's genetic variations on drug efficacy and toxicity (74). Colon cancer is a major public health concern, and its treatment involves the use of various chemotherapy drugs. However, the efficacy of these drugs can vary significantly among individuals, due to differences in their genetic profiles (75). Therefore, understanding the pharmacogenomics of colon cancer is crucial for developing personalized treatment plans. One of the most well-known pharmacogenomic biomarkers for colon cancer is the presence of KRAS mutations. The KRAS gene is frequently mutated in colorectal cancer, which has been associated with resistance to EGFR inhibitors like cetuximab and panitumumab (76). These drugs are commonly used to treat metastatic colorectal cancer, and their efficacy is significantly reduced in patients with KRAS mutations. Therefore, testing for the presence of this mutation has become an essential step in selecting the appropriate therapy for colon cancer patients. Another important pharmacogenomic biomarker for colon cancer is the dihydropyrimidine dehydrogenase (DPD) enzyme. DPD is responsible for the metabolism of 5-fluorouracil (5-FU), which is a commonly used chemotherapy drug for colon cancer. Patients with decreased DPD activity are at a higher risk of developing severe toxicities when treated with 5-FU. Therefore, testing for DPD deficiency is recommended before starting 5-FU therapy (77). In addition to KRAS and DPD, several other genetic variants have been associated with the efficacy and toxicity of chemotherapy drugs used in colon cancer. For example, the UGT1A1*28 allele has been linked to an increased risk of severe toxicities when treated with irinotecan (78). Similarly, the TPMT gene variants have been associated with a higher risk of myelosuppression when treated with thiopurine drugs, such as azathioprine and mercaptopurine (79).

Plasma and tissue proteomic biomarkers

Several plasma and tissue proteomic biomarkers have shown promise in early detection and diagnosis of colon cancer (Table 3). Carcinoembryonic antigen (CEA) and epithelial cell adhesion molecule (EpCAM), have shown promise in early detection and diagnosis of colon cancer (80, 81). Elevated levels of CEA have been associated with advanced stages of colon cancer and poor prognosis. EpCAM has been shown to be a potential target for cancer therapy, and its expression levels have been used to predict patient outcomes. Other potential tissue biomarkers for colon cancer include heat shock protein 27 (HSP27), guanine nucleotide-binding protein subunit beta-2-like 1 (GNB2L1), and peroxiredoxin-1 (PRDX1) (82, 83). Tryptophan metabolism dysregulation has been linked to colorectal tumorigenesis, and altered levels of tryptophan metabolites and indole derivatives contribute to the promotion of tumorigenesis by altering the immune response, inducing inflammation, and affecting the balance of gut microbiota. Studies have shown that tryptophan is inversely associated with colon cancer risk (84, 85). According to a study conducted by Vasaikar and colleagues, the use of proteomics has revealed a correlation between reduced infiltration of CD8 T cells and heightened glycolytic activity in microsatellite instability-high (MSIH) tumors. This finding indicates that targeting glycolysis may serve as a potential strategy to overcome the resistance of MSI-H tumors to immune checkpoint blockade (86). A proteomics study by Tang et al. showed that proteins like TFR1, SAHH, and HV307 were differently expressed in colon cancer patients (87). Several studies have demonstrated that tryptophan and indole metabolism pathways are dysregulated in colon cancer, leading to altered levels of tryptophan metabolites and indole derivatives. These metabolic changes contribute to the promotion of tumorigenesis by altering the immune response, inducing inflammation, and affecting the balance of gut microbiota (88). Zhong et al. showed that extracellular vesicles containing SPARC and LRG1 were differentially expressed in colon cancer patients as compared to healthy subjects, and differed by tumor location (89).

Plasma and tissue metabolomics and microbiomics

Recent advancements in molecular profiling techniques, such as metabolomics and microbiomics, have provided new insights into the pathogenesis and progression of colon cancer (Table 4). Metabolomics and microbiomics focus on the comprehensive analysis of small molecules and microbial communities, respectively, in biological samples. Metabolomics analysis can provide a comprehensive understanding of the metabolic alterations that occur during colon cancer development and progression. The analysis of plasma metabolites in colon cancer patients has shown alterations in amino acid, lipid, and carbohydrate metabolism. These metabolic alterations are related to cancer cell proliferation, invasion, and metastasis. In addition, metabolomics analysis of colon tumor tissue has revealed significant differences in metabolic pathways, including glycolysis, tricarboxylic acid cycle, and pentose phosphate (105). A study by Deng et al. reported that plasma metabolomic profiling can be helpful in distinguishing left-sided colon cancer from right-sided colon cancer (106). Microbiomics is the study of the microbial communities that reside within a host. The one residing in the intestine, the so-called gut microbiome, is composed of trillions of microorganisms, including bacteria, viruses, fungi, and archaea. Recent studies have shown that the gut microbiome plays a critical role in colon cancer development and progression. The gut microbiome can influence the host's

Table 3. Summary of protein biomarkers in colon cancer, their type, technique used, and sample.

References	Protein Biomarker	Up-/down-regulated	Type of Biomarker	Technique	Sample
Zhang et al. (90)	Transgelin-2 (TAGLN2)	Upregulated	Diagnostic biomarker	Fourier transform mass spectrometry (FTMS)	CRC tissue
Ghazanfar et al. (91)	ACTBL2	Upregulated	Diagnostic biomarker	Two-dimensional gel electrophoresis coupled to mass spectrometry (2DE-MS)	CRC tissue
Hao et al. (92)	DPEP1	Upregulated	Diagnostic biomarker	FTMS	CRC tissue
Jonsson et al. (93)	MMP-9	Upregulated	Diagnostic biomarker	ELISA	CRC tissue
Quesada-Calvo et al. (94)	OLFM4, KNG1, Sec-24	Upregulated	Diagnostic biomarker	Liquid chromatography-mass spectrometry (LC-MS)	FFPE CRC tissue
Guo et al. (95)	PCBP1	Upregulated	Predictive biomarker	2D gel electrophoresis	Cell lines and tumoral tissue
Yamamoto et al. (96)	Cyclophilin A, Annexin A2, Aldolase A	Upregulated	Diagnostic biomarker	LC-MS	FFPE CRC tissue
Zhang et al. (97)	LRG1	Upregulated	Diagnostic biomarker	Quantitative real-time PCR and ELISA	CRC tissue and plasma
Katsila et al. (98)	pEGFR	Upregulated	Predictive biomarker	Quantitative proteomic analysis	Plasma
Ivancic et al. (99)	LRG1, EGFR, ITIH4, SOD3	Upregulated	Diagnostic biomarker	Targeted liquid chromatography-tandem mass spectrometry	Serum
Bhardwaj et al. (100)	MASP1, Osteopontin, PON3, TFRC1, Amphiregulin	Upregulated	Diagnostic biomarker	Liquid chromatography	Plasma
Langers et al. (101)	MMP-2, MMP-9	Upregulated	Predictive biomarker	ELISA	CRC tissue
Yu et al. (102)	STK4 or MST1	Downregulated	Diagnostic biomarker	Mass spectrometry (MS/MS), ELISA	Serum
Martin et al. (103)	APOE, AGT, DBP	Upregulated	Predictive biomarker	Gel electrophoresis (2D-DIGE) followed by LC-MS/MS	Serum
Yang et al. (104)	PSMA1, LAP3, ANXA3, serpin B5	Upregulated	Predictive biomarker	Mass spectrometry	Serum and CRC tissue

immune system, metabolism, and gut barrier function, all of which are critical factors in colon cancer pathogenesis. In colon cancer patients, there is a significant dysbiosis in the gut microbiome, with a reduction in beneficial bacteria and an increase in harmful bacteria. Moreover, certain bacterial species have been implicated in colon cancer development and progression, such as *Fusobacterium nucleatum, Streptococcus bovis,* and *Escherichia coli* (107, 108). These bacteria can promote tumorigenesis by inducing inflammation, altering the gut microenvironment, and producing genotoxic metabolites.

Tailored therapy

Despite advances in diagnosis and treatment, a significant proportion of patients with colon cancer continues to experience disease recurrence and treatment resistance. Tailored therapy, which involves individualizing treatment based on a patient's unique characteristics, has emerged as a promising approach to improving outcomes in colon cancer. Tailored therapy in colon cancer involves the use of biomarkers, which are molecular or cellular indicators that can predict disease behavior or response to therapy (116). These biomarkers can be used to identify subgroups of patients who are likely to respond to specific treatments or who have a higher risk of disease recurrence. Some of the most commonly used biomarkers in colon cancer include microsatellite instability (MSI), KRAS mutation status, and BRAF mutation status. Patients with MSI-high colon cancer are more likely to respond to immune checkpoint inhibitors, which activate the immune system to attack cancer cells (117). In contrast, patients with MSI-low or microsatellite-stable (MSS) colon cancer may benefit from conventional chemotherapy regimens. KRAS and BRAF mutations are associated with resistance to certain chemotherapy drugs, and patients with these mutations may benefit from alternative treatment approaches. For example, patients with KRAS mutations may benefit from targeted therapies that inhibit downstream signaling pathways, such as EGFR inhibitors (118). Similarly, patients with BRAF mutations may benefit from combination therapies that target multiple signaling pathways (119).

Table 4. Summary of metabolomics and microbiomics biomarkers for early diagnosis of colon cancer.

Author	Metabolite(s)/Pathway (s)	Up-/Down-regulated	Specimen	Findings
Cross et al. (109)	Glycochenodeoxycholate	Upregulated	Serum	Positive association was observed in CRC among women
Long et al. (110)	Xanthine, hypoxanthine, D-mannose	Downregulated	Serum	Lower levels observed in CRC compared to control. Hypoxanthine to xanthine levels indicative of CRC progression
Sinha et al. (111)	Clostridia Lachnospiraceae p-aminobenzoate and conjugated linoleate	Downregulated	Feces	A strong correlation was observed in fecal samples of CRC patients. Metabolites predominated by Enterobacteriaceae and Actinobacteria.
	Fusobacterium Porphyromonas p-hydroxy-benzaldehyde palmitoyl-sphingomyelin	Upregulated		
Gao et al. (112)	Methionine Tyrosine Valine Isoleucine	Upregulated	Tissue	Tissue amino acid profile has good sensitivity and specificity for diagnosis of CRC ($p<0.001$)
Ning et al. (113)	Glycolysis and amino acid metabolism	Upregulated	Urine	GC-TOF/MS-based metabolomics as diagnostic biomarkers for CRC
	Lipid metabolism	Downregulated		
Wang et al. (114)	Choline Phenylalanine Asparagine Isocitrate Cysteine Hippurate Dimethyl sulfone Creatinine Alanine Methylamine	Downregulated	Urine	NMR-based urinary metabolomics has potential for early diagnosis of CRC
	Homocysteine Glutamine cis-Aconitate Acetoacetate trans-Aconitate Guanidoacetate	Upregulated		
Yang et al. (115)	Proline and Glutamine	Upregulated	Feces	Microbe-associated metabolites have diagnostic potential for CRC
	Glycerol Linoleic acid Oleic acid	Decreased		
	Proteobacteria Fusobacteria Firmicutes	Upregulated		

Conclusions

Colon cancer is a complex and heterogeneous disease that requires a multi-omics approach for its proper diagnosis, prognosis, and treatment. The integration of omics sciences and precision medicine has revolutionized our understanding of colon cancer and the way we approach diagnosis and treatment. The identification of specific genetic mutations and genomic rearrangements, as well as the development of new biomarkers have allowed for more accurate diagnoses and personalized treatment plans. This information can guide the selection of tailored therapies, which can improve patient outcomes and reduce treatment-related toxicities. Precision medicine based on omics technologies is rapidly advancing in the field of colon cancer, and its potential to revolutionize cancer care treatment cannot be overstated. By identifying the specific molecular abnormalities driving cancer growth and progression, precision medicine can offer targeted therapies that can block or inhibit those abnormalities. The ability to tailor therapy based on each patient's characteristics has led to improved patient outcomes and better overall survival rates. However, there is still much to be learned in this field, and continued research is necessary to identify new biomarkers and therapeutic targets. Furthermore, access to these cutting-edge technologies must be expanded, to ensure that all patients can benefit from personalized treatment plans.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Omics sciences and precision medicine in sarcoma

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Abstract

Background. Sarcomas are a relatively rare but diverse group of cancers that typically develop in the mesenchymal cells of bones and soft tissues. Occurring in more than 70 subtypes, sarcomas have broad histological presentations, posing significant challenges of prognosis and treatment. Modern multi-omics studies, which include genomics, proteomics, metabolomics, and micro-biomics, are vital to understand the underlying mechanisms of sarcoma development and progression, identify molecular biomarkers for early detection, develop personalized treatment plans, and discover drug resistance mechanisms in sarcomas to upsurge the survival rate.

Aim. This study aims to highlight the genetic risk factors responsible for sarcoma-genesis, and to present a comprehensive review of multi-omics studies about sarcoma.

Methods. Extensive literature research was undertaken using reliable and authentic medical journals, e-books, and online cancer research databases. Mendelian inheritance in man database (OMIM) was explored to study particular genes and their loci that are responsible to cause various sarcomas.

Result. This in-depth research led to the finding out that omics studies provide a more comprehensive understanding of underlying molecular mechanisms of sarcomas. Through genomics, we can reveal genetic alterations that predispose to sarcoma, like mutation in TP53, NF1, and so on. Pharmacogenomics enable us to find molecular targets for specific drugs. Whereas, proteomic and metabolomic studies provide insights into the biological pathways involved in sarcoma development and progression.

Conclusion. Future advancements in omics sciences for sarcoma are on the cutting-edge of defining precision treatment plans and improved resilience of sarcoma patients. ***Clin Ter 2023; 174 Suppl. 2 (6):68-76 doi: 10.7417/CT.2023.2473***

Key word: *sarcoma, omics sciences, genomics, genetics, metabolomics, microbiomics, proteomics, cancer*

Introduction

Sarcoma is a histologically diverse group of malignant tumors that develop in connective tissues of fat, bones, cartilage, and muscles (1). Predominantly, sarcomas originate in mesenchymal stromal cells (MSC) of the bone marrow, which are undifferentiated stem cells, causing osteoblasts, adipoblasts, chondroblasts, and several other connective tissue cells (2).

Sarcomas are referred to as 'rare' tumors because of their low incidence rates. Until recently, annual occurrence of sarcoma is <6/100,000 individuals worldwide (1,3,4). The majority of sarcoma sufferers are young children (accounting for >20% of total pediatric), whereas only 1% of all older adults malignancies are connected to sarcoma (1,4).

To date, there have been about 100 different types of sarcomas described in the 2020 WHO classification of tumors, each of which is etiologically and pathologically distinct in said publication (3,5). This new classification has upgraded clinical decision-making, correct pathological diagnosis, surveillance and management of this heterogenous cancer (5). For the sake of convenience, scientists segregated sarcomas in three broader classes, namely: bone sarcoma, soft tissue sarcoma (STS, which occurs in muscles, nerves, retroperitoneum, blood vessels, originating mostly in extremities), and visceral sarcoma (occurring in specific organs, like the gastrointestinal tract) (4,6). Among these multiple varieties, the majority of diagnosed sarcoma originate in soft connective tissues, mostly in extremities (60% of cases), with over 50 histological subtypes, whereas 10% of sarcoma subtypes belong to bone sarcoma (4,5).

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Although the exact etiologies and causes of many STSs are not well-understood, certain risk factors have been recorded to increase the risk of sarcomas. Environmental risk factors like exposure to ionizing radiation, carcinogenic chemicals (like arsenic, anabolic steroids, and thorium), virus infection, prior tumor experience, increased body mass index, age, diabetes, and obesity are associated with a higher risk of developing sarcoma (4,6,7). Genetic anomalies responsible to cause STSs include the inactivation of tumor suppressor gene because of germline mutations, like in neurofibromatosis and nerve sheath tumors. Sarcoma genesis may occur due to hereditary genetic predisposition of genes, like in Li-Fraumeni-syndrome (6,7).

The discovery of diagnostic biomarkers and treatment targets for cancers is indispensable, but identifying the basic tumor driving forces, like genomic alterations, and their impact on sarcoma phenotypes remain major challenges. "Omic" sciences are helping in this regard, as they provide high-throughput approaches to assess metastasis phenotypes and chemotherapy resistance and to find therapeutic targets. Genomics, metabolomics, transcriptomics, and proteomics research can lead to a better insight into the oncogenesis, improved prognosis, and personalized tumor management (8).

Clinical presentation and diagnosis of sarcoma

Identical to the diverse nature of sarcoma, the clinical presentation of each STS patient is highly variable. Sometimes a lump or a mass appears on bones and extremities, and every so often sarcoma may remain unnoticed. Rare typical symptoms of STSs include fever, weight loss, and weakness (9).

Because of STSs' rarity and histologic overlaps, diagnosing them is a great deal. Primary diagnosis is done using medical imaging (CT scan, MRI) combined with biopsy investigations from the affected tissue (9)(4). In addition to conventional topography and morphology-based strategies, molecular pathogenetic testing has revolutionized STS diagnosis. Staging of STS is done on the basis of 'grades'. This grading system has been suggested by the French Federation of Cancer Centers Sarcoma Group (FNCLCC), which is also approved by WHO (4,10). Grades are best to describe necrosis and mitotic activity in cancers, and serve as powerful prognostic tools to predict metastasis in STS (4,10).

Molecular genetic analyses—like fluorescence in situ hybridization, reverse transcription polymerase chain reaction, and sequencing of targeted genes—are advancements in routine diagnosis of STS (11). Moreover, genomic screening and high-throughput targeted sequencing have broadened the landscape of precision oncology of sarcomas and its therapeutic goals (12). Although histopathological findings serve as the backbone of STS diagnosis, the potential utility of recent molecular profiling and omics information through immunohistochemical markers also improves pathological diagnosis of STS (11). These methods make it easier to find new therapeutic targets to formulate personalized medical care for STS (12,13).

Genetic susceptibility of sarcomas: cancer predisposition syndromes

Identifying genetic causes of sarcomas is still difficult, because most STS types—such as liposarcoma, synovial sarcoma, angiosarcoma, and Ewing sarcoma—occur sporadically (14,15). However, Mendelian Randomization (MR) approaches to elucidate underlying causal associations of STS with familial gene segregations have been documented in many studies and case reports. MR is the analysis of germline genetic variants, like single nucleotide polymorphisms (SNPS), and has an edge over other conventional observational studies. It helps to specifically identify risk factor of interest by measuring the genetic variability, independent of other biological pathways, that is randomly assigned at conception (16).

Somatic cell mutations happen after meiosis and are confined to cancerous cells, while germline mutations occur in all the cells of an organism. When cancers are observed in families with consistent pattern (Mendelian genetic pattern), they are regarded as having familial cancer predisposition syndrome. Various sarcomas are attributed to arise from heritable cancer predisposition syndromes (14,15).

Familial cancer predisposition syndromes significantly contribute to premature mortality (17). Those who survive after STSs are at an increased risk of developing any other form of cancer. So, it is advisable to turn the testing of germline variants that predispose to sarcoma into a routine clinical practice for entire suspected families and individual members diagnosed with STS. This will help in grasping the disease's natural condition and the patients' therapeutic needs, thus tailoring their personalized treatments. Also, it will enable the family members to seek genetic counselling and figure out their cancer risk (15).

A study highlighted two germline pathways that are mainly responsible to cause mesenchymal cancers (18). One involves the variation in centrosome genes during a mitotic division; the other is the heritable variations in shelterin complex (six telomer-associated proteins). This study confirms the findings of prior studies, showing that pathogenic variants occurring in mitosis and telomeres are heritable and enriched in STS patients (17,18).

There has been a longstanding association between sarcoma and cancer predisposition syndromes. Most of the pathogenic germline variants of CPGs are observed in TP53, NF1 and BRCA1/2 genes (15,17). Here, we are briefly discussing some selected heritable cancer predisposition syndromes that elicit sarcomas. An overview of the genes causing various sarcomas has been summarized in Table 1 below (14)(19):

1. **POT1 tumor predisposition (POT1-TPD)**
   It develops when a heterozygous pathogenic variant POT1is identified in a proband by molecular genetic testing. It increases the risk of developing multiple cutaneous melanomas, gliomas, chronic lymphocytic leukemia, and, particularly, angiosarcoma. First-degree relatives of suspected patients should also be tested for POT1-TPD, as it is genetically transferred as an autosomal dominant disorder. Each offspring of such patients would have 50%-increased chances of getting POT1-TPD phenotypic spectrum (20).

Table 1. Selected inherited genetic syndromes with genes that cause sarcomas (14)(19)

Inherited Syndromes	MIMs of syndrome phenotypes	Genes	Cytogenetic location	Gene OMIMs	Emerging Sarcoma	Inheritance
APC, Gardner syndrome (Familial adenomatous polyposis)	175100	APC	5q22.2	611731	Desmoid tumors	AD
Beckwith-Wiedemann syndrome	130650	ICR1, KCNQ1OT1, CDKN1C	11p15.5, 11p15.5, 11p15.4	616186, 604115, 600856	Embryonal rhabdomyosarcoma (RMS)	AD/ sporadic
Bloom	210900	RECQL3	15q26.1	604610	Osteosarcoma, Embryonal RMS	AR
Carney-Stratakis	606864	SDHB, SDHC, SDHD	1p36.13, 1q23.3, 11q23.1	185470, 602413, 602690	GIST	AD
Constitutional mismatch repair syndrome	619101	PMS2	7p22.1	600259	Embryonal RMS	AR
Costello	218040	HRAS	11p15.5	190020	Embryonal RMS	AD
Familial GIST	606764	SDHB, SDHC, KIT	1p36.13, 1q23.3, 4q12	185470, 602413, 164920	GIST	AD
Familial pleuropulmonary blastoma (DICER1 syndrome)	601200	DICER1	14q32.13	606241	Embryonal RMS	AD
Familial rhabdoid predisposition syndrome	609322	SMARCB1	22q11.23	601607	Malignant rhabdoid tumor	AD/ somatic
Gorlin syndrome/nevoid basal cell carcinoma syndrome	109400	PTCH2, PTCH1, SUFU	1p34.1, 9q22.32, 10q24.32	603673, 601309, 607035	Embryonal RMS	AD
Hereditary retinoblastoma	180200	RB1	13q14.2	614041	Osteosarcoma, STS	AD
HLRCC	150800	FH	1q43	136850	Uterine leiomyosarcoma	AD
Li Fraumeni Syndrome	151623	TP53	17p13.1	191170	Osteosarcoma, RMS, STS	AD
Mosaic variegated aneuploidy	257300	BUB1B	15q15.1	602860	Embryonal RMS	AR
Multiple osteochondromas	133700	EXT1	8q24.11	608177	Chondrosarcomas	AD
Neurofibromatosis 1	162200	NF1	17q11.2	613113	MPNST, GIST, RMS	AD
Nijmegen breakage syndrome	251260	NBN	8q21.3	602667	Embryonal RMS	AR
Noonan syndrome	163950	PTPN11	12q24.13	176876	Embryonal RMS, giant cell tumor of bone, granular cell tumor, PVNS	AD
Rothmund-Thomson syndrome- II	268400	RECQL4	8q24.3	603780	Osteosarcoma	AR
Rubinstein-Taybi	180849	CREBBP	16p13.3	600140	Embryonal RMS, LMS	AD
Tuberous sclerosis	191100	TSC1	9q34.13	605284	PEComa tumor (Pacoima), chordomas	AD
Werner syndrome	277700	RECQL2	8p12	604611	Osteosarcoma, embryonal RMS	AR

Abbreviations: *AD, autosomal dominant; APC, adenomatous polyposis coli; AR, autosomal recessive; GIST, gastrointestinal stromal tumor; HLRCC, hereditary leiomyomatosis and renal cancer; LMS, leiomyosarcoma; MPNST, malignant peripheral nerve sheath tumor; NF1, neurofibromatosis type1; PEComa, perivascular epitheloid cell tumor; RCC, renal cell carcinoma; RMS, rhabdomyosarcoma; STS,*

2. NTHL1 tumor syndrome

It develops in patients who are diagnosed with germline biallelic pathogenic variant in NTHL1. Diagnosis is made using molecular genetic testing, and it runs genetically as autosomal recessive manner. It increases the risk of getting colorectal cancer, breast cancer, and colorectal polyposis. It is advisable to check relatives, even those who are asymptomatic, for early diagnosis and appropriate treatment (21).

3. **Rhabdoid tumor predisposition syndrome**
   Malignant rhabdoid tumors develop when mutation occurs in SMARCB-1 or SMARCA4 genes. It increases the risk of rhabdoid tumors, which are malignancies mainly of the nervous system ad brain, but they can also occur at any anatomical location. It predominantly develops in infants before three years of age. It is genetically transferred in autosomal dominant manner. Pathogenic germline variant SMARCB1 has de novo disease causing ability and can be diagnosed without a family history of such tumors (22).
4. **DICER1 tumor predisposition**
   Mutation in germline DICER1 pathogenic variant leads to an increased risk of developing pleuropulmonary blastoma, thyroid gland neoplasia, thyroid cancer, ovarian tumors, and cystic nephroma. Occasionally, it may lead to cause rhabdomyosarcoma and central nervous system sarcoma. It is inherited in autosomal dominant fashion, and relatives should be screened to overcome future pathogenic circumstances. Early detection and genetic counselling are keys to surveillance (23).
5. **Li-Fraumeni syndrome (LFS)**
   This is an inherited condition or cancer predisposition syndrome that increases the chances of getting various types of childhood- and adult-onset tumors in an individual. Five commonly observed cancers in Li-Fraumeni syndrome are osteosarcoma, soft tissue sarcoma, adrenocortical carcinomas, breast cancer, and central nervous system cancers. The risk of developing cancer with LFS is greater in females (>90%) than in males (>70%).
   The variant that is responsible to cause LFS is the occurrence of a mutation in TP53 gene, and it is inherited in autosomal dominant fashion in an individual whose family members have experienced an array of various cancers in a single ancestral line (24,25). As TP53 pathogenic variant loses its tumor suppression, leading to malignancy and toxicity, there is also evidence suggesting that genomes that are enriched in functional copies of TP53 may halt the development of cancer (14).
6. **Lynch syndrome**
   This cancer predisposition syndrome increases the risk of developing colorectal cancer and cancers of biologically vital organs, like stomach, ovary, bowel endometrium, urinary tract, skin, brain, pancreas, biliary tract, and sarcomas. Lynch syndrome is the result of a mutation in genes MLH1, MSH2, MSH6, PMS2, and EPCAM. It is inherited in autosomal dominant fashion. Lynch syndrome encompasses a spectrum of different sarcoma types, such as fibrous histiocytomas, rhabdomyosarcomas, liposarcoma, and leiomyosarcomas (25,26).
7. **Hereditary diffuse gastric cancer**
   It is an autosomal dominant disorder, caused by alteration in CDH1 pathogenic variant. It increases the risk of developing diffuse gastric cancer. It is an adenocarcinoma, characterized by infiltration and thickening of the stomach wall without any grown mass or lesion. Another rare gastric tumor may be predisposed as a result of pathogenic variation in PRKAR1A in Carney complex or Carney syndrome. This gastrointestinal stromal tumor was previously termed as "gastric epitheliod leiomyosarcoma" (25).
8. **Neurofibromatosis type 1 or NF1**
   NF1 disorder is one of the most commonly occurring human predisposition syndromes, with a frequency of 1 in 3,000 individuals. Prominent features of NF1 are the growth of numerous nerve sheath tumors (called neurofibromas) and marks of cutaneous hyperpigmentation at various sites (called café-au-lait spots). Sometimes axillary freckling, bone dysplasia, optic glioma, and iris hamartomas may also be clinical findings. Pathogenic variant NF1gene—responsible for producing the tumor suppressor neurofibromin—loses its functionality, leading to spontaneous mitogenic signaling via mitogen-activated protein kinase pathways (14,27).
   NF1 disorder increases the susceptibility to develop a highly aggressive soft-tissue sarcoma called malignant peripheral nerve sheath tumor (MPNST), which accounts for 2% of all STS. NF1 syndrome may also give rise to another mesenchymal tumor, the gastrointestinal stromal tumors (GISTs), in the interstitial cells of Cajal present at the lining of GIT. Children affected by NF1are also at greater risk of developing rhabdomyosarcoma (14,27).
9. **Familial adenomatous polyposis (FAP)**
   Principally, it is a colorectal cancer syndrome that can be identified with numerous adenomas, also called polyps, throughout the lining of the large bowel. These polyps have great potential to transform into a sarcoma called fibromatosis (28). FAP is inherited as autosomal dominant syndrome, resulting from germline alteration in adenomatous polyposis coli (APC) gene. APC is a tumor suppressor that is responsible to regulate the levels of β-catenin, which controls mitogenic signaling. FAP runs in families with 100% penetrance, and patients are likely to develop colorectal cancer after the age of 40. FAP patients predominantly predispose to other complex cancers called desmoid tumors (14).
   Desmoid tumors are fibroblastic neoplasms that usually occur in abdominal walls, mostly in the peritoneum. They are locally aggressive, but rarely metastasize. Their severity is variable, from painless lesions to greatly invasive tumors that can lead to mortality. FAP patients are 15 % more likely to develop desmoid tumors (14,29).
   The correspondence between distinct sarcomas with different heritable cancer predisposition syndromes is naturally complex. Exploring these associations is the center of interest for the formulation of personalized oncologic therapeutics.

Genomics of sarcoma: unraveling of Pharmacogenomics (PGx) biomarkers

Due to the complex genetic origin and histological intricacies of sarcoma, its prognosis is generally poor, and devising effective drugs for its treatment poses significant challenges. Primarily, localized STS is treated by surgically removing the cancer mass, with subsequent radiotherapy, while tumor progression and metastasis are controlled via chemotherapeutics. Nevertheless, significant results to encounter diverse forms of STSs were not achieved, and the survival rate after metastatic STS is as low as 30% after two years of treatment. The reason is the difference

in pharmacological response to drugs, which is unique in every patient (30).

Molecular mechanisms that cause sarcoma initiation

In literature, there are three fundamental molecular mechanisms that have been observed to cause sarcoma-genesis. First of all, malfunctions of gene expression that occur due to anomalous, chimeric transcription factors, resulting from characteristic gene fusions in translocation-associated sarcomas. Secondly, mutations that occur in vital signaling pathways can also propel sarcoma. Lastly, aberrations in DNA copy-number (31).

Novel research on sarcoma is uncovering important genetic information and identifying specific point mutations occurring along with translocations, oncogenes that are lineage-specific, events that remodel chromosomes, and genetic alterations that affect normal signaling and differentiation pathways (31).

Pharmacogenomic (PGx) biomarkers discovery and their clinical application

As each patient's genetic make-up is unique, it will produce a different idiosyncratic response to multifactorial drugs, which can be tricky to foretell. Polymorphic variants in genes responsible for drug absorption, distribution, metabolism, and excretions (ADME) influence the pharmacokinetics (PK) and pharmacodynamics (PD) of a drug. These PKs and PDs intervene at different biological levels, including metabolome, epigenome, transcriptome, and proteome, giving distinct clinical outcomes. Such unpredictable drug responses, due to polymorphic variants in genes, directly affect drug dosage, efficacy, toxicity, chances of hypersensitivity, and drug resistance (32).

Pharmacogenomics (PGx), sometimes called pharmacogenetics, have been utilized in genetics with the ultimate goal of identifying 'genomic variations' that can offer valuable insights for enhancing drug effectiveness and minimizing the risks of chemotherapy-related side effects (33). Numerous studies and clinical trials have shown interindividual unique responses to certain drugs.

Recently, the correlation between common genetic alterations and drug responses have been explored and published widely for large cohorts of individuals from diverse populations, and are included in the Genome Wide Association (GWAS) catalog, generated by the National Human Genome Research Institute (NHGRI) and the European Bioinformatics Institute (EMBL-EBI) (32). Genomic profiling has led to developing specialized pharmacogenomic biomarkers that help to foresee specified drug responses and figure out genetic basis, possible risks of toxicity, and differences in treatment efficacy for STS patients (30,34).

The germline alterations in the genome of patients, especially single nucleotide polymorphisms (SNPs), are highly penetrant predisposed mutations that serve as the potential biomarkers for drug-induced toxicity and drug response. Also, cancer predisposing somatic mutations that occur randomly due to DNA damage have been impeccably utilized as drug targets (30,34). These pharmacogenomic (PGx) biomarkers, which can predict efficacy and any possible adverse drug reactions, are incorporated in membrane transporters, drug targets, drug-metabolizing enzymes, and HLA alleles (30,34).

Here, we are selectively discussing some valuable pharmacogenomic biomarkers, that have been extensively implicated and studied for STS patients.

– The human solute carrier family (SLC) are membrane transport proteins responsible to carry inorganic ions, lipids, neurotransmitters, amino acids, and drugs. From this family of membrane transporters, organic cations transporter (OCTs) and nucleoside transporters (NTs) have been widely studied for STS, suggesting that OCT6-mutation may have long lasting effects on PKs and PDs of doxorubicin in breast cancer patients (34,35).

– Human equilibrative nucleoside transporter or h-ENT1 also belong to SLCs, and is found in erythrocytes, brain, placenta, mammary glands, and other soft tissues. It is a nucleoside transporter that influences the absorption of pyrimidine-based drugs like gemcitabine (34). A retrospective study, performed on leiomyosarcoma and angiosarcoma patients, was carried out to understand the link between hENT1 expression and clinical response of gemcitabine: it showed that high levels of hENT1 are linked with better clinical results of gemcitabine in sarcoma patients (34).

– ATP-binding cassette (ABC) superfamily consists of seven different classes of membrane proteins that are involved in multi-drug resistance; thus, they can cause cancer treatment failure by efflux of antineoplastic molecules. In this family, ABCB, ABCC, and ABCG subtypes are widely studied for sarcomas. ABCB encodes p-glycoprotein (Pg-p), whose expression levels are found remarkably high in many tumor cells—including STS, breast, gastric, kidney, leukemic, and liver cancers. Conventional drugs used for the treatment of STS include anthracyclines, taxanes, and tyrosine kinase inhibitors like imatinib and sorafenib. Their responses are significantly affected by high levels of Pg-p (34,36).

– ABCC family consists of six pumps, with abundance of MRP1, which maintains cellular resistance to anthracyclines; MRP2 reduces oral absorption and enhances hepatobiliary clearance of drugs. A study performed on 60-year-old male STS patients showed that SNPs were prominent in ABCC family and adverse events with trabectedin (34,37).

– Pyrimidine metabolism is a key component of DNA/RNA, important for phospholipids and protein metabolism, and also serves as a target of many chemotherapeutic regimens. Its antagonists—like 5-fluorouracil, gemcitabine, and cytarabine—have been shown noticeable success by inhibiting its synthetic enzymes, like nucleoside monophosphate kinase (UMPK/CMPK). CMPK plays an important role in the synthesis of cytidine analogs and is the potential target of gemcitabine-based chemotherapies for leukemia, solid tumors, and lymphoma. (34).

– Cytochrome P450 family, a group of oxidative enzymes that are claimed to metabolize anti-cancer drugs, exhibiting remarkable variations in its genes (CYP). A study was conducted to observe the effects of cyclophosphamide in rhabdosarcoma patients: results showed that carrying mutant CYP2B6 alleles affected the patient's response to cyclophosphamide. Patients who carried only one mutation

(in particular SNP of CYP2B6) showed better impression of the drug, while patients with three alleles of mutant SNPs exhibited short event-free survival (34).

– Breast cancer 1 gene (BRCA1) encodes for tumor suppressor protein. It works in response to DNA damage, and mutations in this gene indicate hereditary breast and ovarian cancers. It also serves as an important biomarker for sarcoma, which has been noted to give improved prognosis and disease management for sarcoma patients. Increased expression of BRCA1 is linked with lower response to trabectedin (34,38).

– Role of sodium Dichloroacetate (SDA) as an anti-tumor agent

Anti-oxidant enzymes and pro-oxidant processes perform key functions in the development of sarcoma. A study measured the activities of anti-oxidant enzymes in sarcoma-affected tissue homogenates of mice that are treated with SDA. Results showed that SDA minimized the activities of oxidative enzymes playing key roles in tumorigenesis, thus demonstrating anti-cancer effects of SDA (39).

A well-known property of cancer, including sarcomas, is the presentation of Warburg effect, in which the glycolytic pathway is extraordinarily activated even in the presence of oxygen, leading to enhanced tumor growth (40). This aerobic glycolysis is an inefficient way for ATP synthesis to fulfill the demands of uncontrolled proliferating cells, thus creating an imbalance of nutrients and energy for somatic cells. To overcome or reverse this Warburg effect, dichloroacetate (DCA) is widely utilized to treat sarcoma, which is a pyruvate dehydrogenase kinase (PDK) inhibitor, since it potentially restricts tumor growth and reduces the resulting apoptosis. Research and clinical trials have verified the efficacy of this treatment in managing sarcoma (41).

Immuno-pharmacogenomics

Immunomodulatory pathways in sarcomas can be potential targets for immunotherapy, in combination with tailored therapy. Next generation sequencing methods are utilized for genetic profiling of host immune cells in the emerging field of immunogenomics or immune-pharmacogenomics, also revealing their potential to enhance immunotherapy efficacy, as well as to serve as a mediator for the activity of cytotoxic agents and targeted drugs. This new approach holds promise for providing valuable information to predict clinical outcomes and to monitor the treatment response of sarcoma. Moreover, it may help identify tumor neoantigens that could be targeted for novel immunotherapies (42).

For instance, Poly (ADP-ribose) polymerase-1 inhibitors (PARP1-i) are responsible to activate immunomodulatory pathways in STS cells and also to alter tumor microenvironment. For this reason, PARP1-i is an attractive candidate to combine with immune checkpoint inhibitors (ICI), which will ultimately improve efficacy and provide effective therapy for tumors. Likewise, T-cells of upgraded affinity have also been transferred in the patients (especially in synovial sarcoma) for tumor-specific antigens and have given promising results (34).

For planning customized treatments for the patients and to sidestep unwanted toxicities of drugs, it is highly necessary to identify and develop absolute pharmacogenomic biomarkers with the goal to maximize therapeutic benefits, as the genuine success of getting personalized medicine for sarcoma patients is associated with the discovery of pharmacogenomic biomarkers that will help to stratify patients as responsive and non-responsive subjects. It will also make it possible to find out whether the patients have chances to develop treatment-associated toxicity (33).

Metabolomics of sarcoma

Metabolomics of biomolecules facilitate the study of metabolite concentrations and their widespread outcomes in metabolic pathways, reflecting the associations between the genotype and the phenotypes of a cell. The implication of metabolomics in sarcoma has enabled the researchers to identify the involvement of specific metabolic pathways and their alterations in the development and progression of these tumors. Such studies of different types of sarcomas revealed distinct metabolic signatures associated with each subtype (8). Furthermore, applications of this tactic also provided insights into the mechanisms of drug resistance in sarcomas, as well as helped identify potential therapeutic targets (43).

Cell cycle deregulation in sarcomas occurs due to deviations from major metabolic pathways, like glycolysis, biosynthesis of macromolecules (like amino acids and nucleotides), and mitochondrial respiration. Major oncogenic alterations include boosted glycolysis, glutaminolysis, and oxidative phosphorylation in sarcomas, increasing ATP production and establishing chemoresistance. As a whole, STS display overexpression of tyrosine kinase receptor (Her4) and activation of RAS, PI3K, and HIPPO pathways, coupled with a predominant glycolytic/oxidative phosphorylation signature. The cancer genome atlas (TCGA) database reveals unique metabolic profiles in individual STS subtypes as compared to other cancers. For instance, enhanced PPAR/fatty acid and glycine/serine/threonine pathways are features of UPS subtypes, whereas increased OXPHOS levels are observed in LMS subtypes (44).

Glucose metabolism in sarcoma

Glucose homeostasis is crucial to be maintained by two pathways, named glycolysis and gluconeogenesis (45). Fructose bis-phosphatase (FBP) is an important rate-limiting enzyme of these pathways, converting fructose 1,6-bisphosphate to fructose-6-phosphate and inorganic phosphate. It is extensively found in muscles and mesenchymal cells. The role of fructose-1,6 bis-phosphatase (FBP) is reported in sarcoma—its loss being a key event in sarcoma development—and it is an active target for anti-sarcoma drugs (46).

Numerous studies corroborated that cancer cells, including STS, exhibit the ubiquitous feature of metabolizing glucose through Warburg effect in times of biosynthetic requirements of nutrients. In tumors, glucose uptake is remarkably enhanced by aerobic glycolysis, in which, instead

of making pyruvate as an end product and transporting it to mitochondria, cancer cells switch glycolysis cycle to make lactate, which is released in extracellular matrix (45). This leads to an acidic microenvironment that facilitates tumor growth and invasiveness, also decreasing mitochondrial activity, which can cause oxidative stress and DNA damage. The key regulating enzyme of Warburg effect, lactate dehydrogenase-A (LDH-A), may be a promising target of therapies in certain types of STS that exhibit overexpression of LDHA (44,45).

Amino acid metabolism

Various amino acids (like glutamine, arginine, and tryptophan) have been studied in the metabolomics of STS. Predominantly, glutamine and arginine metabolic pathways are found to promote STS progression (44). Glutamine is metabolized by mitochondrial glutaminase (GLS), a rate-limiting enzyme of glutaminolytic pathway. Its activity is examined in different STS subtypes, including undifferentiated pleomorphic sarcoma (UPS), synovial sarcoma, and leiomyosarcoma, which showed elevated protein expression of GLS in these tumors (45). Therefore, GLS inhibitors have also been experimented in vivo and in vitro, demonstrating the upsurge of cell cycle arrest, reduction in cell proliferation, and augmentation in cell death, thus showing dependency of tumor cells on glutamine metabolism (46). Similarly, arginine metabolism in sarcoma showed that sarcoma cells lack the rate-limiting enzyme of this semi-essential amino acid, which is required for its synthesis. Tumor cells thus become arginine deficient, leading to starvation and metabolic stress (46).

Presently, many studies have reported the potential of integrated genomic and metabolomic stratagems for interpretation of tumor complexity. A recent article from The Cancer Genome Atlas (TCGA) research network proposed a new classification STS subtypes by combining genetic, epigenetic, and transcriptomic analyses. They evaluated copy number variations (CNVs) and identified three dominant profiles from leiomyosarcoma (LMS), myxofibrosarcoma (MFS), and undifferentiated pleomorphic sarcoma (UPS) that exhibited the maximum number of genomic alterations. Moreover, epigenetic apparatus, by activating pathways and immune signatures, complements prognostic value (44).

Several studies evidenced that STS growth affects mitochondrial fitness. As it depends upon the availability of key metabolites, glycolysis, glutaminolysis and oxidative phosphorylation, disruption of various mitochondrial pathways occurs. For example, in major STSs, a disturbance of TCA cycle occurs, due to the alterations in the expression of its enzymes, such as SDH and FH, and to the excess of oncometabolites. Eventually, it leads to tumor chemoresistance, poor prognosis, and heterogeneity in mitochondrial activity (44).

Proteomics of STS

Research suggests that a proteome holds more than 1000-folds cellular information than the genome, which is transcribed to more than 100,000 transcripts and gives rise to millions of protein variants or protein isoforms because of alternative splicing (AS), single amino acid polymorphisms (SAPs), and wide-ranging post-translational modifications (PTMs). Thus, proteome is a promising field to study the cellular systems in carcinomas, having the potential to reveal valuable biomarkers and drug targets (47).

Proteomics involve the whole protein fractions of a cell and address post-translational modifications (PTMs) and protein-protein associations. Next-generation drug discovery approaches are aimed to target protein inactivators, such as proteolysis targeting chimeras (PROTACs) and immune-oncology agents. Proteomic tools consists of mass spectrometry and protein array methodologies to measure myriads of PTMs in proteome and diverse physiochemical properties of amino acids (48).

In a study, tyrosine phosphorylation (pY) signaling pathways were observed in sarcoma subtypes. 10 different histologically distinct subtypes are studied including osteosarcoma, rhabdomyosarcoma, leiomyosarcoma, and Ewing's sarcoma. Authors of this study identified some exceptional phosphorylation proteins, platelet derived growth factor receptor alpha (PDGFR), and some other unique signaling proteins, whose expression was found elevated in distinct sarcoma subtypes. Some other studies also experimented the identification of phosphoprotein biomarkers, which are helping in patient stratification and prognosis (46).

Microbiomics of sarcoma

These days, microbiome interactions with immune system have gained much importance in defining antitumor immune responses. The reason is that microbiome (genes of microorganisms, along with cellular environment entities) plays a crucial role in the progression of tumors, and gut microbiome configuration affects the clinical outcomes of immune checkpoint-blockade therapy. A growing body of research suggests that an intratumoral microbiome exists, since it was observed that in many cancers it contributes to cancer advancement and interferes with immunotherapy responses (49).

A recent study by Perry et al. (2023) detected the viral microbiome of an intratumoral soft tissue sarcoma, for the first time in a prospective STS cohort utilizing a firmly sterile collection protocol. They found a unique and noticeable microbiome, namely a *Respirovirus*, in tumor samples of STS patients, that exhibited reproducibility and correlation with natural killer cells infiltration. They observed a complex interaction between tumor microenvironment (TME), host immune system, and intratumoral microbiome. In general, natural killer (NK) cells, the activators of the innate immune system, are able to target both cancerous and virally infected cells upon stimulation. Herein, viral microbiome is linked with higher NK cells infiltration inside the tumor, which may provide a useful link for prognosis. This may also contribute to the provision of potential therapeutic target in immunotherapy resistant STS patients. The viral microbiome was found in high levels in patients with local STS, and low levels in patients with metastasis (49).

In another study, distinct fungal population or mycobio-

me has also been detected in 35 cancer types, mostly found in cancer cells and immune cells. Those fungal ecologies exhibited specificity with age, tumor subtypes, smoking, response to immunotherapy, and survival rate. The authors also detected multiple mycobiome-bacteriome-immunome complexes and determined their co-occurrence in tumors. Categorically, they acknowledged fungi-stimulated pan-cancer mycotypes, with characteristic immune responses that will help to stratify patient survival and clinical outcomes. Firm positive interactions observed between fungal and bacterial varieties, obtained from several cancer types, suggest the existence of multi-domain microbial colonization in tumor microenvironment (50).

Previously, similar findings about the presence of bacterial population have also been reported a few decades ago, in a patient of Kaposi's sarcoma (51). Another study by Xin et al (2022) backed up the hypothesis that micro-organisms favor carcinogenesis. Xin and colleagues found abundant tumor-associated fungal populations in diverse cancer types, which strengthened the notion that fungi are ubiquitous to all major cancers; moreover, their specific type can be predictive of endurance (52).

In the next few decades, multi-genomic studies on sarcoma would be primarily focused on the development of precision medicines, as they rely on identifying candidate genes and their mutations, which are susceptible tumor drivers involved in phenotypic presentations, drug resistance, and metabolic alterations in tumors. Tailored therapy of STS aims to collect omics data about the patient as a whole, and molecular info of developed cancer, and match them with a medicine with highest compatibility and minimal side effects (33). Research on omics biomarkers will lead to early monitoring of recurrence of STS and will also be helpful for finding novel therapeutic targets.

Conclusion

The implication of Omics sciences in sarcoma research holds great promises for improving our understanding of the underlying causes of these tumors, grasping the disease's natural condition and the therapeutic needs of STS patients, thus tailoring a personalized treatment for each of them. Specifically, the links between cancer predisposition genes (CPGs) and sarcomas may be a landmark to early diagnosis, screening, and genetic counselling, thus leading to anticipation strategies for index patient and their family. Not only, studying such links can restrain the increasing risk of malignancies, and can also help in developing personalized medicines for STS patients.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Omics sciences and precision medicine in glioblastoma

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Abstract

Glioblastoma is a highly aggressive and malignant type of brain cancer with a poor prognosis, despite current treatment options of surgery, radiation therapy, and chemotherapy. These treatments have limitations due to the aggressive nature of the cancer and the difficulty in completely removing the tumor without damaging healthy brain tissue. Personalized medicine, using genomic profiling to tailor treatment to the patient's specific tumor, and immunotherapy have shown promise in clinical trials. The blood-brain barrier also poses a challenge in delivering treatments to the brain, and researchers are exploring various approaches to bypass it.

More effective, personalized treatment approaches are needed to improve outcomes for glioblastoma patients. This tumor is studied using genomics, transcriptomics, and proteomics techniques, to better understand its underlying molecular mechanisms. Recent studies have used these techniques to identify potential therapeutic targets, molecular subtypes, and heterogeneity of tumor cells.

Advancements in omics sciences have improved our understanding of glioblastoma biology, and precision medicine approaches have implications for more accurate diagnoses, improved treatment outcomes, and personalized preventive care. Precision medicine can match patients with drugs that target specific genetic mutations, improve clinical trials, and identify individuals at higher risk for certain diseases. Precision medicine, which involves customizing medical treatment based on an individual's genetic makeup, lifestyle, and environmental factors, has shown promise in improving treatment outcomes for glioblastoma patients. Identifying biomarkers is essential for patient stratification and treatment selection in precision medicine approaches for glioblastoma, and several biomarkers have shown promise in predicting patient response to treatment. Targeted therapies are a key component of precision medicine approaches in glioblastoma, but there is still a need to improve their effectiveness.

Technical challenges, such as sample quality and availability, and challenges in analyzing and interpreting large amounts of data remain significant obstacles in omics sciences and precision medicine for glioblastoma. The clinical implementation of precision medicine in glioblastoma treatment faces challenges related to patient selection, drug development, and clinical trial design, as well as ethical and legal considerations related to patient privacy, informed consent, and access to expensive treatments. ***Clin Ter 2023; 174 Suppl. 2 (6):77-84 doi: 10.7417/CT.2023.2474***

Key words: *glioblastoma, omics sciences, genomics, metabolomics, proteomics, diagnosis, therapy, precision medicine*

Introduction

Brief overview of glioblastoma and its high mortality rate

Glioblastoma is a type of brain cancer that arises from glial cells, which are supportive cells in the brain. It is the most aggressive and malignant type of brain cancer, with a median survival time of only 15 months since the diagnosis, even with aggressive treatments. The exact etiology of glioblastoma is unknown, but it is believed to be linked to genetic mutations and abnormal growth of glial cells. Symptoms of glioblastoma may include headaches, seizures, memory loss, personality changes, and difficulty in speaking or movements. Treatment options include maximal safe surgical resection, radiation therapy and concomitant chemotherapy, followed by adjuvant chemotherapy. However, the prognosis of patients affected by glioblastoma remains poor regardless of the type of treatment adopted.

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The high mortality rate of glioblastoma is due to several factors, including tumor aggressiveness, challenges to achieve gross total resection preserving neurological functions, and the limited effectiveness of current treatments. According to the National Brain Tumor Society, glioblastoma is the most common primary malignant brain tumor, accounting for about 47% of all primary brain tumors. The five-year survival rate for glioblastoma is less than 10%, and only about 5% of patients survive for 10 years after the diagnosis (1).

Research is ongoing, to better understand the underlying causes of glioblastoma and to develop more effective treatments. One promising approach involves the use of personalized medicine, which uses the patient's genetic information to tailor treatment to their specific tumor. Clinical trials are currently underway to test the safety and effectiveness of personalized medicine for glioblastoma (2, 3).

Current standard treatment options for glioblastoma and their limitations

The standard treatment options for glioblastoma include surgery, radiation therapy, and chemotherapy. These treatments can help slow down the progression of the disease, but they have several limitations.

Surgery is the first-line treatment option for glioblastoma and is aimed to accomplish the maximal tumor resection. However, since glioblastoma tends to infiltrate surrounding brain tissue, it can be difficult to remove the entire tumor without causing postoperative neurological deficits (4).

Radiation therapy on the other hand, uses high-energy beams of radiation, and while it can be effective in slowing the growth of glioblastoma, it can also damage healthy brain tissue, leading to side effects such as fatigue, headaches, and cognitive problems (5, 6).

Finally, chemotherapy involves the use of drugs to kill cancer cells. It can be effective in slowing down the progression of glioblastoma, but can be limited by the occurrence of significant side effects, including headache, nausea, vomiting, fatigue and pancytopaenia. Additionally, the blood-brain barrier can limit the amount of chemotherapy drugs that reach the brain, thus making it difficult to reach therapeutic levels.

Newer treatment approaches that are currently being investigated for glioblastoma include targeted therapy, immunotherapy, and gene therapy. However, these approaches are still in the early stages of development and have not yet been proven to be effective in clinical trials (7).

The need for more effective, personalized treatment approaches

Glioblastoma remains a challenging tumor to treat and cure. Therefore, there is a need for more effective, personalized treatment approaches.

One promising path for personalized treatment is the use of genomic profiling to identify the specific genetic mutations and abnormalities driving the growth of the tumor. This information can be used to target those specific mutations with targeted therapies or immunotherapies. It was found that glioblastoma patients who received targeted therapies based on genomic profiling had improved outcomes as compared to patients who received standard treatments (8-10).

Immunotherapy, already adopted to treat other cancers, has shown promising results in early clinical trials for glioblastoma. Furthermore, patients with recurrent glioblastoma who underwent experimental immunotherapy had a longer median survival (12 months) than patients who received standard treatments (5.5 months) (11, 12). Apart from personalized treatments, more effective pharmacologic strategies to cross the blood-brain barrier and deliver drugs to the tumor are needed. Ultrasounds and nanotechnologies are under investigation to overcome this obstacle (13).

Omics Sciences in Glioblastoma

Genomics techniques used in glioblastoma research

Genomics techniques are essential tools for understanding the underlying molecular mechanisms of glioblastoma. For example, whole genome sequencing (WGS) and whole exome sequencing (WES) can be used to identify genetic mutations and copy number variations in glioblastoma. Moreover, RNA sequencing (RNA-seq) can be used to identify differentially expressed genes and potential biomarkers, while single-cell sequencing can enable the identification of heterogeneity and clonal evolution in glioblastoma. Finally, DNA methylation analysis is used to identify epigenetic changes in glioblastoma. These techniques have enabled the identification of potential therapeutic targets and the development of precision medicine approaches for glioblastoma treatment. However, technical challenges exist in the analysis and interpretation of the large amounts of data generated by these techniques. Variability in sample quality and availability also poses a significant challenge in glioblastoma research. One study used WGS to identify somatic mutations in glioblastomas and found that the mutations were associated with specific molecular subtypes of the disease, which could help guide personalized treatments (14). Another study used genomic analysis to identify a genetic alteration that drives resistance to the temozolomide, which is the first-line chemotherapy drug used to treat glioblastoma (15).

Transcriptomics techniques used in glioblastoma research

Transcriptomics is the study of gene expression at the transcript level. This technique can provide valuable information about the underlying molecular mechanisms of glioblastoma and can help in biomarker identification. Several transcriptomics techniques have been used in glioblastoma research, including microarray analysis, RNA-seq, and single-cell RNA sequencing (scRNA-seq). Microarray analysis allows for the measurement of the expression levels of thousands of genes simultaneously, and has been used to identify differentially expressed genes in glioblastoma as compared to normal brain tissue. RNA-seq provides higher resolution and sensitivity than microarray analysis and has been used to identify novel spliced transcripts and fusion genes in glioblastoma. scRNA-seq allows for the identification of heterogeneity within tumors and has been used to identify subpopulations of cells with different gene

expression profiles. These transcriptomics techniques have provided valuable insights into the molecular mechanisms of glioblastoma and have the potential to identify novel therapeutic targets. Transcriptomic analysis was used in a study to identify a set of genes that are overexpressed in glioblastomas and could serve as potential therapeutic targets (16). Another study used transcriptomics to identify a molecular signature associated with poor survival in glioblastoma patients (17).

Proteomics techniques used in glioblastoma research

Proteomics techniques are essential tools for understanding the protein expression and post-translational modifications, finally resulting in biomarkers and therapeutic targets identification. They can be applied also to study glioblastoma tumorigenesis and progression. One widely used technique is mass spectrometry, which can identify and quantify thousands of proteins in a single sample. Additionally, proteomics approaches can also be used to study protein-protein interactions, protein localization, and protein function.

Other proteomics techniques include two-dimensional gel electrophoresis, which separates proteins based on their isoelectric point and molecular weight, and protein microarrays, which allow for high-throughput analysis of protein expression and interactions. These techniques have been used to identify potential biomarkers and therapeutic targets in glioblastoma, such as the upregulation of EGFR and the downregulation of PTEN in tumor tissues. However, challenges remain in standardizing proteomics protocols and interpreting the large amounts of data generated from these techniques. One study used proteomic analysis to identify a protein that is overexpressed in glioblastomas and could serve as a therapeutic target (18). Another study used proteomics to identify proteins that are differentially expressed in response to treatment with temozolomide (19).

Examples of recent studies using these techniques

A number of studies used genomics, transcriptomics, and proteomics techniques to further our understanding of glioblastoma.

Darvin and colleagues used RNA-seq to identify the subset of patients who may benefit from treatment with immune checkpoint inhibitors. They found that patients with high levels of immune-related gene expression had better survival outcomes after treatment with immune checkpoint inhibitors (20).

In another study, the researchers used genomic and transcriptomic analysis to identify a novel molecular subtype of glioblastoma, called MES-IG, characterized by high levels of immune and inflammatory signaling. They found that MES-IG tumors were more responsive to immune checkpoint inhibitors than other subtypes of glioblastoma (21).

Bollard and co-workers identified a set of proteins upregulated in glioblastoma and associated with poor outcomes by means of proteomic analysis. They demonstrated how targeting one of these proteins, called PIM1, could improve treatment outcomes in glioblastoma patients (22).

Eventually, researchers used scRNA-seq to analyze the heterogeneity of glioblastoma tumors at the single-cell level. They found that glioblastomas are highly heterogeneous tumors and different subpopulations of tumor cells may respond differently to treatment (23).

Advancements in omics sciences have improved our understanding of glioblastoma biology

The genomics of glioblastoma has been extensively studied to identify mutations and alterations that drive tumor's growth and progression. The Cancer Genome Atlas (TCGA) project identified, on the basis of genomic alterations, four molecular subtypes of glioblastoma with different prognoses and response to therapies. The IDH1 mutation was found to

Table 1. List of genes involved in glioblastoma, with OMIM id, the pathology to which they are correlated, and the inheritance pattern.

Location	Phenotype	Inheritance	Phenotype MIM number	Gene/Locus	Gene/Locus MIM number
2q34	Glioma, susceptibility to, somatic	-	137800	IDH1	147700
5p15.33	Glioma susceptibility 8	-	613033	GLM8	613033
7q31.33	Glioma susceptibility 9	AD	616568	POT1	606478
8q24.21	Glioma susceptibility 7	-	613032	CCDC26	613040
9p21.3	Glioma susceptibility 5	-	613030	GLM5	613030
10q23.31	Glioma susceptibility 2	-	613028	PTEN	601728
13q13.1	Glioblastoma 3	AR	613029	BRCA2	600185
15q23-q26.3	Glioma susceptibility 4	-	607248	GLM4	607248
17p13.1	Glioma susceptibility 1	AD, SMu	137800	TP53	191170
17q12	Glioblastoma, somatic	-	137800	ERBB2	164870
20q13.33	Glioma susceptibility 6	-	613031	GLM6	613031

be a strong prognostic factor for glioblastoma patients (24). Transcriptomic analyses have been used to identify genes that are differentially expressed in glioblastoma, which can serve as potential therapeutic targets or biomarkers. The TCGA project identified the EGFR signaling pathway as a key pathway activated in glioblastoma. Some drugs targeting this pathway have been developed and tested in clinical trials (25).

Epigenetic alterations, such as DNA methylation and histone modifications, have been shown to play a role in glioblastoma development and progression. Epigenetic changes can alter gene expression and promote tumor growth. Epigenetic therapies have been developed and tested in clinical trials (26). Moreover, proteomic analyses have been used to identify proteins that are differently expressed in glioblastomas and can serve as potential therapeutic targets or biomarkers (27).

Precision Medicine in Glioblastoma

Definition of precision medicine and its use in glioblastoma treatment

Precision medicine refers to the customization of medical treatment based on individual's genetic makeup, lifestyle, and environmental factors. In glioblastoma, precision medicine involves identifying specific genetic mutations that drive tumor growth and developing targeted therapies to inhibit these mutations. Precision medicine approaches have shown promise in improving treatment outcomes for glioblastoma patients (28).

Importance of identifying biomarkers for patient stratification and treatment selection

Identifying biomarkers is essential for patients' stratification and treatment selection in precision medicine approaches. Biomarkers are molecular indicators of disease or response to treatments, and their identification can help match patients with the most effective therapies. In glioblastoma, identifying biomarkers is particularly important due to the heterogeneity of the disease and the variable response to treatment (29).

Examples of promising biomarkers in glioblastoma

Several biomarkers have been identified in glioblastoma, including *IDH1* and *IDH2* mutations, MGMT promoter methylation status, and *EGFRvIII* mutations, or alteration of PI3K/AKT/MTOR and RAS/RAF/MEK/MAPK signaling pathways, DNA-damage repair pathways and cell cycle checkpoints. These biomarkers can help predict patients' response to treatments and guide treatments' selection (30,31).

Targeted therapies in precision medicine for glioblastoma

A key component of precision medicine approaches in glioblastoma are targeted therapies. These therapies selectively target specific molecular pathways that drive tumor growth and survival, leading to improved treatment outcomes. Examples of targeted therapies in glioblastoma include EGFR inhibitors, VEGF inhibitors, MEK/BRAF inhibitors, or recently IDH1 inhibitors as vorasidenib (32).

Overview of current FDA-approved targeted therapies for glioblastoma

There are currently few FDA-approved targeted therapies for glioblastoma, including regorafenib (a multikinase inhibitor). However, these therapies have limited efficacy, and there is a need for more effective targeted therapies in glioblastoma (33).

Implications for precision medicine approaches

There are several implications of precision medicine approaches, including more accurate diagnoses, improved treatment outcomes, and the potential for personalized preventive care. Precision medicine can help clinicians make more accurate diagnoses by identifying genetic mutations that contribute to a patient's disease. For example, genetic testing can help diagnose cancer and guide treatment decisions (34,35). Moreover, precision medicine can also help identify the most effective treatments for patients by matching them with drugs that target specific genetic mutations. Precision medicine can improve clinical trials by selecting patients with specific genetic profiles that are more likely to respond to a particular treatment. This can help speed up drug development and reduce the number of patients needed for clinical trials (35,36). Precision medicine can also be used to identify individuals who are at higher risk for certain diseases, thus developing personalized preventive care plans. For example, genetic testing can identify individuals at higher risk for hereditary cancers, allowing for earlier screening and intervention (37).

Challenges and Limitations of Omics Sciences and Precision Medicine in Glioblastoma

Technical challenges in omics sciences for glioblastoma research

Glioblastoma is a complex and heterogeneous disease, and omics sciences have become essential tools for understanding its underlying molecular mechanisms. However, several technical challenges of omics sciences need to be addressed to improve glioblastoma research.

A review discusses the challenges of single-cell sequencing technology in glioblastoma research, including data quality control, data analysis, and interpretation (38). Another article highlights the need for standardized protocols in proteomics research to reduce variability and increase reproducibility (39). Eventually, a study identifies several challenges in the analysis of DNA methylation data in glioblastoma research, such as normalization and batch effect removal (40).

Challenges in analyzing and interpreting large amounts of data

Omics sciences generate huge amounts of data, that are challenging to be analyzed and interpreted. The integration of multi-omics data in glioblastoma research, such as data normalization, feature selection, and machine learning algorithms, can be reached with difficulties (41). Similarly, RNA-seq data in glioblastoma, such as the identification of novel biomarkers and the validation of differential gene expression, are difficult to be interpreted (42).

Variability in sample quality and availability

Obtaining high quality and sufficient quantity of samples for omics sciences is a significant challenge in glioblastoma research. Tumor tissue heterogeneity and necrosis are limiting factors for genomic analyses (43), such as rarity of some brain tumors for sample availability (44) and sample preservation and processing for proteomics research (44,45).

Clinical challenges in implementing precision medicine in glioblastoma treatment

Precision medicine holds promise for improving glioblastoma treatments, but its clinical implementation faces several challenges. Patients' selection, drugs' development, clinical trial design (46), identification of possible molecular targets, development of effective combination therapies (47), integrating genomic data into the clinical decision-making for the treatment of glioblastomas (48) are just some of these challenges.

Ethical and legal considerations in using precision medicine for glioblastoma patients

The use of precision medicine in glioblastoma treatment raises ethical and legal considerations. There are several ethical challenges posed by using genomic data in clinical decision-making, concerning also the potential impact on patient's autonomy and privacy (49). Other issues include data privacy, intellectual property, and informed consent (50,51). Furthermore, ethical considerations of providing expensive precision medicine treatments to patients with limited access to healthcare resources should be taken into account (52).

Future Directions and Conclusion

Promising developments in omics sciences and precision medicine for glioblastoma

Omics sciences and precision medicine have the potential to revolutionize glioblastoma treatment. A review article highlights the promising developments in omics sciences, including single-cell sequencing, spatial transcriptomics, and liquid biopsy analysis (53). Another article discusses the potential of precision medicine in glioblastoma treatment, such as the identification of novel biomarkers and the development of targeted therapies (54).

Ongoing targeted therapies for glioblastoma

Several clinical trials are currently ongoing to identify targeted therapies for glioblastoma. For example, a clinical trial is evaluating the safety and efficacy of a combination therapy, consisting of Toca 511 (a retroviral replicating vector) and Toca FC (a prodrug of the antifungal drug 5-fluorocytosine), for patients with recurrent high-grade glioma, including GBM. The therapy works by selectively targeting cancer cells and delivering a cytotoxic drug to kill them. The trial is currently ongoing (at phase 3) and has shown promising results so far (55).

AG-881 is a dual inhibitor of the metabolic enzymes isocitrate dehydrogenase-1 and 2 (IDH1/2), which are commonly mutated in GBM. The drug has been evaluated in phase 3 clinical trial for patients with IDH-mutant recurrent or progressive GBM. The trial aims to determine whether AG-881 can improve overall survival compared to standard chemotherapy (56).

Regorafenib is a multikinase inhibitor, whose main targets are kinases involved in angiogenesis (VEGFR1–3 and TIE2), oncogenesis (KIT, RET, RAF1, and BRAF), tumor microenvironment (PDGFR and FGFR), and tumor immunity (colony stimulating factor 1 receptor). The administration of regorafenib in patients with recurrent glioblastoma has shown encouraging results (57,58).

Another possibility is the use of immune checkpoint inhibitors, such as pembrolizumab and nivolumab, which have shown promising results in the treatment of several cancers (including GBM). These drugs work by blocking proteins expressed by cancer cells that inhibit the immune system's ability to attack them. Moreover, a potential linkage with immunotherapy and PARP inhibitors has been identified in 44% of glioblastoma patients as a consequence of alterations in DNA-damage repair genes, supporting the purpose of their combination in clinical setting. Several clinical trials are ongoing to evaluate the safety and efficacy of these drugs in combination with other therapies for GBM (59).

The potential impact of omics sciences and precision medicine on glioblastoma treatment and patient outcomes

Omics sciences and precision medicine have the potential to significantly impact glioblastoma treatment and patient outcomes. Via advanced technologies and personalized approaches, precision medicine can potentially lead to more effective and targeted treatments. For instance, the identification of specific genetic mutations in glioblastoma tumors can inform the use of targeted therapies, such as EGFR inhibitors or IDH inhibitor (60). Additionally, omics sciences can aid in the identification of novel biomarkers for diagnosis and monitoring of glioblastoma (61).

Furthermore, the use of precision medicine may also lead to better patient outcomes and survival rates. A study found that patients with IDH-mutant glioblastomas had a significantly better overall survival rate when treated with IDH-targeted therapies as compared to standard chemotherapy (62). Another study showed promising results for the use of the PARP inhibitor talazoparib in glioblastoma patients with DNA damage response gene mutations (63). Overall, omics sciences and precision medicine hold signi-

ficant potential for improving glioblastoma treatment and patient outcomes.

Conclusion and recommendations for future research and clinical practice

In conclusion, glioblastoma is a highly aggressive and deadly form of brain cancer, and its treatment remains a significant challenge. However, recent advances in omics sciences and precision medicine offer promising opportunities for improved diagnosis and treatment. Future research in glioblastoma should focus on addressing the technical challenges in omics sciences, such as sample variability and data analysis. Additionally, there is a need for ongoing clinical trials to evaluate the efficacy and safety of emerging targeted therapies, as well as to identify biomarkers that can predict treatment response. Furthermore, it is important to address the clinical challenges in implementing precision medicine in glioblastoma treatment, such as patient stratification and treatment selection. This requires collaboration among clinicians, researchers, and patients to develop personalized treatment plans that consider the unique characteristics of each patient's tumor. Finally, there is a need to address ethical and legal considerations in using precision medicine for glioblastoma patients, such as ensuring privacy, informed consent, and access to expensive treatments. By addressing these challenges, omics sciences and precision medicine can potentially improve glioblastoma treatment and patient outcomes. Overall, the future of glioblastoma research and clinical practice will rely on multidisciplinary collaboration and a personalized approach to treatment that considers the unique characteristics of each patient's tumor.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Omics sciences and precision medicine in pancreas cancer

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Abstract

Pancreatic cancer is a leading cause of death worldwide, associated with poor prognosis outcomes and late treatment interventions. The pathological nature and extreme tissue heterogeneity of this disease has hampered all efforts to correctly diagnose and treat it. Omics sciences and precision medicine have revolutionized our understanding of pancreatic cancer, providing a new hope for patients suffering from this devastating disease. By analyzing large-scale biological data sets and developing personalized treatment strategies, researchers and clinicians are working together to improve patient outcomes and ultimately find a cure for pancreatic cancer. ***Clin Ter 2023; 174 Suppl. 2 (6):85-94 doi: 10.7417/CT.2023.2475***

Key words: *Pancreas cancer, precision medicine, biomarker, genomics, metabolomics*

Introduction

Pancreatic cancer is a highly aggressive malignancy with a poor prognosis (1). It is the fourth leading cause of cancer-related deaths in the United States, with a five-year survival rate of less than 10% (2). It is often diagnosed at an advanced stage, making it difficult to treat (3). Recent advances in omics sciences and precision medicine have opened up new avenues for early detection and personalized treatment (4-6); but, despite the advances in medical technology and treatment options, the five-year survival rate for pancreatic cancer is still low (7). However, there is hope in the fight against this deadly disease, thanks to the emergence of precision medicine (8).

Precision medicine involves tailoring treatments to an individual's unique genetic makeup, thus allowing for more targeted and effective therapies (9). The pancreas is an extremely important part of the human body, for its digestive as well as regulatory roles: in fact, it produces enzymes and hormones through different cellular pathways. Pancreas contains acinar cells, duct cells, and the islets of Langerhans, which are involved in various functions by producing cell secretions. For instance, acinar cells produce inactive zymogen enzymes, which are activated upon exposure to the bicarbonate-rich pancreatic juice secreted by duct cells. Whereas, the islets of Langerhans are associated with the secretion of insulin and glucagon hormones, regulating glucose concentration in the body.

In this article, we will explore the role of genomics and proteomics in understanding the genetic basis of pancreatic cancer, identifying biomarkers for early diagnosis, and developing targeted therapies that can improve patient outcomes. We will also discuss the challenges and opportunities associated with translating these scientific discoveries into clinical practice. Despite advances in treatment, the disease remains difficult to diagnose and treat. Furthermore, we will explore the latest developments in precision medicine for pancreatic cancer, including targeted therapies and immunotherapies. We will also discuss specific precision medicines—such as Pamrevlumab, Herceptin®, Larotrectinib (LOXO-101), and Erbitux® (cetuximab)—that are showing promising results in clinical trials. By understanding these cutting-edge treatments, we can gain hope for a brighter future in the battle against pancreatic cancer. Moreover, recent developments in omics sciences and precision medicine have provided new hope for patients with pancreatic cancer.

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Prevalence of pancreatic cancer

Pancreatic cancer is one of the deadliest cancers, with a five-year survival rate under 10% (9) and an incidence that has been increasing over the past few decades, projected to become the second leading cause of cancer-related deaths by 2030 (10). The risk factors for pancreatic cancer include smoking, obesity, chronic pancreatitis, diabetes mellitus, and family history (11). Unfortunately, most cases are diagnosed at an advanced stage, when treatment options are limited (2, 12). Therefore, early detection and personalized treatment strategies are crucial for improving patient outcomes (13). Omics sciences such as genomics and proteomics have emerged as powerful tools for identifying novel biomarkers and therapeutic targets in pancreatic cancer (14).

What are omics sciences?

The name "omics sciences" refers to the study of large-scale biological data sets, including genomics, transcriptomics, proteomics, metabolomics, and epigenomics (15). These data sets provide a comprehensive view of the molecular changes that occur in cells and tissues during disease development and progression. By analyzing these data sets, researchers can identify new targets for therapy and develop personalized treatment strategies for patients.

Genetics of the pancreatic tumor

Pancreatic cancer is a highly aggressive and lethal malignancy, with poor prognosis. The genetic alterations that occur in pancreatic cancer are complex and heterogeneous, involving multiple genes and signaling pathways (16, 17). Several studies have identified specific genetic mutations associated with the development of pancreatic cancer, including KRAS, TP53, CDKN2A, SMAD4, and BRCA2. KRAS mutations are the most common genetic alteration found in pancreatic cancer, occurring in up to 95% of cases (18). These mutations lead to constitutive activation of the RAS signaling pathway, which promotes cell proliferation and survival (19). TP53 mutations are also frequently observed in pancreatic cancer and are associated with increased tumor aggressiveness and resistance to therapy (20). CDKN2A mutations result in loss of function of the p16INK4a protein, which regulates cell cycle progression and is involved in DNA repair mechanisms (21). SMAD4 mutations impair TGF-β signaling pathway activity, leading to increased cell proliferation and invasion (22). Finally, BRCA2 mutations have been linked to an increased risk of developing pancreatic cancer (23). Overall, understanding the genetic alterations that occur in pancreatic cancer is critical for developing targeted therapies that can improve patient outcomes. Advances in genomics technologies have enabled researchers to identify novel gene biomarkers that may be useful for early detection or predicting response to treatment.

Genomics of pancreatic tumor

Pancreatic cancer is a complex disease that arises from the accumulation of genetic and epigenetic alterations (24). Genomic studies have provided insights into the molecular mechanisms underlying pancreatic cancer development and progression. Whole-genome sequencing, whole-exome sequencing, and transcriptome analysis have identified numerous genetic alterations in pancreatic tumors, including mutations in KRAS, TP53, CDKN2A, SMAD4, and other genes (25-27).

The most frequently mutated gene in pancreatic cancer is KRAS, which is mutated in more than 90% of cases (25, 28). The KRAS mutation leads to constitutive activation of the RAS signaling pathway, which promotes cell proliferation and survival (29). Collins et al. studied that pancreatic cancer is associated with mutations in the KRAS gene, but it was not fully understood how these mutations promote cancer (30). Two mouse models were used to study pancreatic tumorigenesis, and it was found that KRAS mutations are required for tumor cell survival and promote the formation and maintenance of the fibroinflammatory stroma that plays a pivotal role in pancreatic cancer. Inhibiting KRAS mutations could provide a new approach for the treatment of pancreatic cancer.

Other commonly mutated genes include TP53 (50-75%), CDKN2A (30-40%), and SMAD4 (20-30%) (31). These mutations are associated with poor prognosis and resistance to therapy (32). For instance, Sinn et al. used next generation sequencing (NGS) in CONKO-001 trial to identify prognostic and predictive mutations in pancreatic adenocarcinoma patients (33). TP53 mutations were found to be a negative prognostic factor for disease-free survival in untreated patients but a positive predictive factor for gemcitabine efficacy in treated patients. Bartsch et al. evaluated the prevalence of mutations in the CDKN2A gene in familial pancreatic cancer (FPC) and determined their association with malignant melanoma (34). Germline mutations in p16 were found to be rare in FPC families, but they were identified in families with both pancreatic cancer and melanoma. No p14 germline mutations were found. These findings suggest a possible pancreatic cancer-melanoma syndrome associated with CDKN2A germline mutations affecting p16, and all members of such families should be screened for these mutations.

In addition to these recurrent mutations, genomic studies have identified other genetic alterations that may contribute to pancreatic cancer development and progression. For example, amplification of MYC or ERBB2 has been observed in a subset of pancreatic tumors (32, 35). Moreover, genomic profiling has revealed distinct subtypes of pancreatic cancer based on their molecular features. These subtypes may have different clinical outcomes and response to therapy. Overall, genomics studies have provided valuable insights into the molecular mechanisms underlying pancreatic cancer and may facilitate the development of precision medicine approaches for this deadly disease.

Table 1. Genes that are commonly associated with pancreatic cancer.

Gene	Function	Role in pancreatic cancer
KRAS	GTPase, regulates cell growth and differentiation	Activating mutations in KRAS are present in >90% of pancreatic ductal adenocarcinomas (PDACs) (36, 37)
TP53	Tumor suppressor gene, regulates cell cycle and apoptosis	TP53 mutations are present in >50% of PDACs (33)
CDKN2A	Tumor suppressor gene, regulates cell cycle	CDKN2A mutations are present in ~95% of familial pancreatic cancer cases and ~50% of sporadic cases (38)
SMAD4	Tumor suppressor gene, regulates TGF-β signaling pathway	SMAD4 mutations are present in ~55% of PDACs (39)
BRCA1/2	DNA repair genes	Germline mutations in BRCA1/2 increase the risk of developing pancreatic cancer (40)
ATM	DNA repair gene	Germline mutations in ATM increase the risk of developing pancreatic cancer (41)
PALB2	DNA repair gene	Germline mutations in PALB2 increase the risk of developing pancreatic cancer (42, 43)
STK11	Tumor suppressor gene, regulates cell polarity and metabolism	Germline mutations in STK11 increase the risk of developing pancreatic cancer (44)

Gene biomarkers of pancreatic cancer

Early detection and accurate diagnosis are crucial for improving patient outcomes. In recent years, significant progress has been made in identifying genetic biomarkers associated with pancreatic cancer (45). Several gene mutations have been identified in pancreatic cancer, including KRAS, TP53, CDKN2A, SMAD4, and BRCA2 (45). The KRAS gene is mutated in about 95% of pancreatic cancer cases, making it one of the most common genetic alterations in this disease. The TP53 gene, involved in regulating cell division and preventing the formation of tumors, has been found mutated in up to 70% of pancreatic cancers. The CDKN2A gene, which encodes a protein regulating cell cycle progression, has been found mutated in up to 95% of pancreatic cancer cases, while the SMAD4 gene, involved in regulating cell growth and division, has been found mutated in up to 55% of them.

Table 2. Some key gene biomarkers associated with prognosis and diagnosis of pancreatic cancer.

Gene	Phenotype MIM number	Pathology Correlation	**Gene/Locus MIM number**	**Inheritance**	**Role in pancreatic cancer**
BRCA2	600185	Increased risk of pancreatic cancer	260350	Autosomal dominant	Germline mutations in BRCA1/2 increase the risk of developing pancreatic cancer (40, 48)
CDKN2A	600160	Increased risk of pancreatic cancer	260350	Autosomal dominant	CDKN2A mutations are present in ~95% of familial pancreatic cancer cases and ~50% of sporadic cases (38, 49)
PALB2	610355	Increased risk of pancreatic cancer	260350	Autosomal recessive	Germline mutations in PALB2 increase the risk of developing pancreatic cancer (42, 43)
ATM	607585	Increased risk of pancreatic cancer	260350	Autosomal recessive	Germline mutations in ATM increase the risk of developing pancreatic cancer (41)
PRSS1	276000	Hereditary pancreatitis	167800	Autosomal dominant	Potential therapeutic target, as PRESS 1 is associated with poor prognosis (50)
STK11	602216	Peutz-Jeghers syndrome (PJS)	175200	Autosomal dominant	Tumor suppressor gene that regulates cell growth and division (51)
MLH1	120436	Lynch syndrome	120435	Autosomal dominant	DNA repair gene; mutations in MLH1 increase risk of developing pancreatic cancer (52)
MSH2	609309	Lynch syndrome	120435	Autosomal dominant	DNA repair gene; works in conjunction with MLH1 to help correct errors that occur during DNA replication (53)
MSH6	600678	Lynch syndrome	120435	Autosomal dominant	DNA repair gene; works in conjunction with MLH1 and MSH2 to help correct errors that occur during DNA replication (54)
PMS2	600259	Lynch syndrome	120435	Autosomal dominant	DNA repair gene; mutations in PMS2 have been associated with an increased risk of developing pancreatic cancer (55)

In short, mutations in these genes are associated with an increased risk of developing pancreatic cancer, which is why these genetic alterations can be used as biomarkers for early detection and prognosis prediction of pancreatic cancer. Furthermore, advances in NGS technologies have enabled the identification of novel genetic biomarkers associated with pancreatic cancer: for example, the GATA6 gene has been identified as a potential diagnostic biomarker for pancreatic ductal adenocarcinoma (PDAC) (46). Additionally, the ARID1A gene has been found to be frequently mutated in PDAC patients with a better prognosis (47).

In conclusion, identifying genetic biomarkers associated with pancreatic cancer can improve early detection and prognosis prediction.

Spatial gene analysis of pancreatic cancer

Spatial gene analysis is a relatively new approach that has been used to study the molecular characteristics of pancreatic cancer (56). This technique allows researchers to analyze the expression of genes in different regions of the tumor, providing valuable information about the heterogeneity of this disease (57). By studying the spatial distribution of genes, scientists have been able to identify specific subpopulations of cells within pancreatic tumors that may be responsible for driving tumor growth and metastasis. One example of spatial gene analysis in pancreatic cancer is single-cell RNA sequencing (scRNA-seq) (58). This technique allows researchers to sequence the RNA from individual cells within a tumor, providing a detailed picture of the gene expression patterns in each cell (59). Using scRNA-seq, scientists have identified distinct subpopulations of cells within pancreatic tumors that exhibit different gene expression profiles (60). These subpopulations may represent different stages of tumor progression or different cell types within the tumor microenvironment. Overall, spatial gene analysis has provided important insights into the molecular mechanisms underlying pancreatic cancer and may help guide the development of more effective treatments for this deadly disease.

Proteomics of pancreatic cancer

Proteomics is the study of proteins and their functions in a cell or organism. In pancreatic cancer, proteomics has been used to identify potential biomarkers for early detection, prognosis, and treatment response (61). Proteomic analysis has also provided insights into the molecular mechanisms underlying pancreatic cancer development and progression (62).

One of the major challenges in pancreatic cancer research is identifying new targets for therapy. Proteomic analysis can help identify novel proteins that are overexpressed or mutated in pancreatic cancer cells as compared to normal cells (62). These proteins can then be targeted with drugs to inhibit their function and prevent tumor growth (63). Furthermore, proteomic analysis can help predict which patients are likely to respond to certain treatments based on the protein expression patterns in their tumors. This information can be used to personalize treatment plans for individual patients, leading to better outcomes. Overall, proteomics is an important tool in understanding the complex biology of pancreatic cancer and developing more effective treatments for this deadly disease.

Pancreatic cancer protein biomarkers

Pancreatic cancer protein biomarkers play a crucial role in the diagnosis and prognosis of pancreatic cancer (64). These biomarkers are proteins that are found in the blood or tissues of patients with pancreatic cancer and can be used to detect the presence of the disease, monitor its progression, and predict patient outcomes (65). One such protein biomarker is CA 19-9, which is commonly used to monitor treatment response and recurrence of pancreatic cancer (66). However, this biomarker has limitations as it can also be elevated in other conditions, such as pancreatitis and liver disease. Other promising protein biomarkers include carcinoembryonic antigen (CEA), carbohydrate antigen 72-4 (CA 72-4), and mesothelin (67-69). These biomarkers have shown potential for early detection of pancreatic cancer and predicting patient outcomes.

For instance, Tempero et al. investigated the relationship between CA19-9 and Lewis antigens in pancreatic cancer patients (70). The researchers found that CA19-9 levels were elevated in patients with pancreatic cancer, and that the presence of Lewis antigen negative status was associated with higher levels of CA19-9. The authors thus suggest that CA19-9 may be a useful biomarker for pancreatic cancer diagnosis and monitoring. Furthermore, Berger et al. examined the use of post-resection CA19-9 levels as a prognostic marker for pancreatic cancer patients undergoing adjuvant chemoradiation (71). The researchers found that higher post-resection CA19-9 levels were associated with worse overall survival in these patients. The authors thus suggest that CA19-9 may be a useful biomarker for predicting outcomes in pancreatic cancer patients following surgery. On this matter, Tzeng et al. investigated racial disparities in CA19-9 levels and pancreatic cancer outcomes (72). The researchers found that Black patients with pancreatic cancer had lower levels of CA19-9 as compared to non-Black patients, and that this difference persisted after controlling for other factors. The authors suggest that these findings highlight the importance of considering race in the interpretation of CA19-9 levels for pancreatic cancer diagnosis and monitoring.

Metabolomics of pancreatic cancer

Metabolomics is the study of small molecules or metabolites present in biological systems. In pancreatic cancer, metabolomics has emerged as a promising tool for identifying biomarkers that can aid in early detection and diagnosis of the disease (86). Metabolomic profiling of pancreatic cancer tissues and biofluids has revealed significant alterations in metabolic pathways, such as glycolysis, amino acid metabolism, and lipid metabolism (86).

One of the key findings from metabolomic studies in pancreatic cancer is the identification of altered levels of certain metabolites, such as glucose, lactate, glutamine, and

Table 3. Some of the protein biomarkers used for prognosis of pancreatic cancer.

Protein Biomarker	**Function**	**Clinical Utility**	**References**
CA19-9	Glycosylated antigen	Diagnosis, Prognosis, Monitoring	(73, 74)
CEA	Glycoprotein	Diagnosis, Prognosis, Monitoring	(67)
MUC1	Glycoprotein	Diagnosis, Prognosis	(75, 76)
Survivin	Inhibitor of apoptosis protein	Diagnosis, Prognosis	(77, 78)
Mesothelin	Glycoprotein	Diagnosis, Prognosis	(79, 80)
Osteopontin	Glycoprotein	Diagnosis, Prognosis	(81, 82)
TIMP1	Protease inhibitor	Diagnosis, Prognosis	(83)
HE4	Glycoprotein	Diagnosis	(84, 85)

fatty acids (87). The researchers identified several metabolites that were significantly different between the two groups, including amino acids, bile acids, and lipids. They found that a combination of four metabolites (valine, leucine, lysine, and taurochenodeoxycholic acid) had high sensitivity and specificity for early detection of pancreatic cancer.

Shen et al. analyzed serum samples from patients with pancreatic cancer and healthy controls using gas chromatography-mass spectrometry (GC-MS) (88). The researchers identified several metabolites that were significantly different between the two groups, including amino acids, fatty acids, and sugars. They found that a combination of four metabolites (lysine, tyrosine, phenylalanine, and palmitic acid) had high diagnostic accuracy for pancreatic cancer. A study was conducted on Chinese cohorts to identify biomarkers for early detection of pancreatic cancer using untargeted metabolomics (89). The study found systematic metabolic network disorders before pancreatic cancer diagnosis, and a novel metabolite panel was identified that may have potential value in early detection of pancreatic cancer.

Another study identified potential metabolite biomarkers for early detection of stage-I pancreatic ductal adenocarcinoma (PDAC) (90). In this work, researchers analyzed the plasma samples of 22 stage-I PDAC patients and 22 healthy controls using gas chromatography-mass spectrometry (GC-MS) and liquid chromatography-tandem mass spectrometry (LC-MS/MS) (90). They identified 46 metabolites that were significantly different between the two groups, including amino acids, lipids, and carbohydrates. The researchers then selected a panel of four metabolites (cysteine, lysine, PC aa C34:3, and PC aa C36:2) as potential biomarkers for early detection of stage-I PDAC. The combination of these biomarkers showed high sensitivity and specificity in distinguishing stage-I PDAC patients from healthy controls. The study suggests that these metabolite biomarkers may have potential for early detection of stage-I PDAC, which could improve patient outcomes. These changes are thought to reflect the metabolic reprogramming that occurs in cancer cells to support their rapid growth and proliferation (91). Metabolomics has also been used to identify potential therapeutic targets for pancreatic cancer by revealing metabolic vulnerabilities that can be exploited for treatment (87).

Precision medicine in pancreatic cancer

Precision medicine is an approach to patient care that takes into account individual variability in genes, environment, and lifestyle (103). In the context of pancreatic cancer, precision medicine involves identifying the specific molecular alterations that drive tumor growth and developing targeted therapies to inhibit these alterations (8). This approach has shown promise in clinical trials, with several targeted therapies showing efficacy in patients with advanced pancreatic cancer.

Pancreatic cancer is a complex disease, requiring personalized treatment options (103). Precision medicine offers a promising approach to treating pancreatic cancer by tailoring therapies to the unique characteristics of each patient's tumor (104). This approach involves analyzing the genetic makeup of the tumor and identifying specific mutations or biomarkers that can be targeted with drugs (105). One example of

Table 3. Some of the commonly reported metabolomics biomarkers in pancreatic cancer.

Metabolite	**Biomarker Type**	**Potential Function**	**Reference**
Glutamate	Diagnostic	Energy production and biosynthesis	(92, 93)
Myo-inositol	Diagnostic	Cell signaling and osmoregulation	(94, 95)
Sphingomyelin	Diagnostic	Membrane structure and cell signaling	(96)
Lysophosphatidylcholine	Diagnostic	Membrane structure and cell signaling	(97)
Creatinine	Prognostic	Muscle metabolism and renal function	(98)
Lactate	Prognostic	Energy metabolism and acid-base balance	(99)
Choline	Prognostic	Membrane structure and cell signaling	(100)
Carnitine	Prognostic	Fatty acid metabolism and energy production	(101)
Glutathione	Prognostic	Antioxidant and detoxification	(102)

precision medicine in pancreatic cancer is the use of PARP inhibitors for patients with BRCA mutations (106). These drugs target a specific DNA repair pathway that is disrupted in tumors with BRCA mutations, leading to cell death (107). Another example is the use of immunotherapy for patients with tumors that express high levels of certain proteins, such as PD-L1 (108). Precision medicine also involves monitoring a patient's response to treatment using liquid biopsies, which can detect circulating tumor DNA in the blood (109). This allows doctors to adjust treatment plans as needed, based on changes in the tumor's genetic profile (110). Precision medicine holds great promise for improving outcomes for patients with pancreatic cancer by providing more targeted and effective treatments (111).

Traditional chemotherapy has been the mainstay of treatment for pancreatic cancer, but it often fails to provide significant benefits due to the heterogeneity of the disease. Precision medicine offers a promising approach to improve outcomes for patients with pancreatic cancer. Precision medicine involves using genomic and molecular information to tailor treatment to an individual patient's unique characteristics (112).

Precision medicine has revolutionized the way we approach cancer treatment, and pancreatic cancer is no exception. Pamrevlumab, Herceptin®, Larotrectinib (LOXO-101), and Erbitux® (cetuximab) are some of the precision medicines that have shown promising results in treating pancreatic cancer (113-115). For instance, Pamrevlumab is a monoclonal antibody that targets a protein called connective tissue growth factor (CTGF), which plays a crucial role in promoting tumor growth and metastasis in pancreatic cancer (113). Clinical trials have shown that combining Pamrevlumab with chemotherapy can significantly improve survival rates in patients with advanced pancreatic cancer (116). Herceptin® is another precision medicine that has been approved for the treatment of HER2-positive breast cancer (117). However, recent studies have shown that HER2 is also overexpressed in a subset of pancreatic cancers (118). This has led to clinical trials investigating the use of Herceptin® in combination with chemotherapy for the treatment of HER2-positive pancreatic cancer (119). Larotrectinib (LOXO-101) is a targeted therapy that inhibits TRK fusion proteins, which are found in a small percentage of pancreatic cancers (120). Clinical trials have shown that larotrectinib can induce durable responses in patients with TRK fusion-positive tumors (120). Erbitux® (cetuximab) is an immunotherapy drug that targets epidermal growth factor receptor (EGFR), which is overexpressed in many types of cancers, including pancreatic cancer (121). Clinical trials have shown that combining Erbitux® with chemotherapy can improve overall survival rates in patients with advanced pancreatic cancer (122).

Targeted therapies in pancreatic cancer

Targeted therapies have shown promising results in treating pancreatic cancer in recent years (110). These therapies work by targeting specific molecules or pathways that are involved in the growth and spread of cancer cells (123).

One example of a targeted therapy is erlotinib, which targets the epidermal growth factor receptor (EGFR) pathway (124). This pathway is often overactive in pancreatic cancer cells, leading to uncontrolled cell growth and division. Another targeted therapy that has shown promise in pancreatic cancer is nab-paclitaxel (125). This drug targets the protein albumin, which is found at high levels in the blood vessels surrounding pancreatic tumors. By binding to albumin, nab-paclitaxel can deliver chemotherapy drugs more effectively, directly to the tumor.

While targeted therapies have shown some success in treating pancreatic cancer, they are not effective for all patients. It is important for physicians to carefully evaluate each patient's individual case and determine which treatment approach will be most effective for them. Ongoing research into new targeted therapies and personalized treatment approaches will continue to improve outcomes for patients with pancreatic cancer.

Immunotherapies in pancreatic cancer

Immunotherapies have emerged as a promising approach in the treatment of pancreatic cancer (126). These therapies work by stimulating the patient's immune system to recognize and attack cancer cells. One type of immunotherapy that has shown promise in pancreatic cancer is checkpoint inhibitors, which block certain proteins on cancer cells that prevent the immune system from attacking them (127). Another type of immunotherapy being investigated for pancreatic cancer is adoptive cell transfer therapy, which involves removing T-cells from a patient's blood and genetically modifying them to target specific cancer cells before infusing them back into the patient's body (128). This approach has shown some success in clinical trials, but more research is needed to determine its effectiveness (129).

Overall, while immunotherapies are still being studied and refined for use in pancreatic cancer treatment, they offer hope for patients who may not respond well to traditional chemotherapy or radiation therapy. As precision medicine continues to advance, it is likely that in the future we will see even more effective immunotherapies, developed specifically for pancreatic cancer.

The future of omics sciences and precision medicine in pancreatic cancer

The future of omics sciences and precision medicine in pancreatic cancer is bright (130): advances in technology have made it possible to analyze large-scale data sets quickly and accurately, allowing for more precise diagnosis and treatment of the disease. In addition, collaborations between researchers and clinicians have led to the development of new clinical trials, incorporating omics sciences and precision medicine approaches (131).

Conclusions

In conclusion, the integration of omics sciences and precision medicine has provided promising approaches to the diagnosis and treatment of pancreatic cancer. Genomics,

spatial gene analysis, proteomics, and metabolomics data have enabled us to identify novel biomarkers and therapeutic targets for this deadly disease. Furthermore, precision medicine approaches—such as targeted therapies and immunotherapies—have shown great potential in improving patient outcomes. However, there is still much work to be done in order to fully understand the complex biology of pancreatic cancer and to develop treatments that are effective for all patients.

Continued research efforts in this field will undoubtedly lead to further advancements in our ability to diagnose and treat pancreatic cancer with greater accuracy and effectiveness. Precision medicine has emerged as a promising approach to treating pancreatic cancer: the ability to tailor treatments based on the unique genetic makeup of each patient has led to the development of targeted therapies and immunotherapies that offer new hope for people suffering from this disease. Pamrevlumab, Herceptin®, Larotrectinib (LOXO-101), and Erbitux® (cetuximab) are just a few examples of precision medicines that have shown promise in clinical trials. While there is still much work to be done in this field, the progress made so far is encouraging and offers hope for better outcomes for patients with pancreatic cancer in the future.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Omics sciences and precision medicine in prostate cancer

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Abstract

In the last decade, Prostate Cancer (PCa) has emerged as the second most prevalent and serious medical condition, and is considered one of the leading factors contributing to global mortality rates. Several factors (genetic as well as environmental) contribute to its development and seriousness. Since the disease is usually asymptomatic at early stages, it is typically misdiagnosed or over-diagnosed by the diagnostic procedures currently in use, leading to improper treatment. Effective biomarkers and diagnostic techniques are desperately needed in clinical settings for better management of PCa patients. Studies integrating omics sciences have shown that the accuracy and dependability of diagnostic and prognostic evaluations have increased because of the use of omics data; also, the treatment plans using omics can be facilitated by personalized medicine.

The present review emphasizes innovative multi-omics methodologies, encompassing proteomics, genomics, microbiomics, metabolomics, and transcriptomics, with the aim of comprehending the molecular alterations that trigger and contribute to PCa. The review shows how early genomic and transcriptomic research has made it possible to identify PCa-related genes that are controlled by tumor-relevant signaling pathways. Proteomic and metabolomic analyses have recently been integrated, advancing our understanding of the complex mechanisms at play, the multiple levels of regulation, and how they interact. By applying the omics approach, new vulnerabilities may be discovered, and customized treatments with improved efficacy will soon be accessible. ***Clin Ter 2023; 174 Suppl. 2 (6):95-103 doi: 10.7417/CT.2023.2476***

Key word: *prostate cancer, omics sciences, genetics, transcriptomics, metabolomics, precision medicine*

Introduction

Prostate Cancer (PCa) is an important medical concern that has a considerable impact on the majority of men population—ranking as the second most prevalent form of malignancy in males (after lung cancer)—and is included in the top five leading causes of mortality globally. In Europe, PCa constitutes approximately 11% of the total male cancers (1), and in the European Union it is responsible for 9% of all cancer-related mortalities in men (2). Risk factors that have been scientifically proven to exist include advanced age, ethnicity, genetics, and family history (3-5). Aside from obesity and physical inactivity, the factors that can contribute to various health conditions for PCa include infections, inflammation, environmental exposures, diet, hyperglycemia, and ionizing radiation (4, 6-10). The initial phases of PCa often exhibit a gradual progression and absence of symptoms, thereby rendering therapy unnecessary. A further progression of the condition may manifest as urinary incontinence and lumbar discomfort (11).

The currently available PCa screening and therapy approaches are invasive and expensive, and frequently result in misdiagnosis or overdiagnosis of the condition; moreover, cancer relapse is quite common. Due to all these associated limitations with screening, increasing incidence rate, and all the risk factors contributing to it, an effective, accurate, non-invasive, and relatively cheaper PCa diagnostic and therapeutic strategy is required. Therefore, the objective of this study is to integrate multi-omics methodologies to better comprehend biomarker discovery and to speed up the adoption of precision oncology in PCa. This review summarizes recent research and highlights some studies that have applied multi-omics to PCa in unique and groundbreaking ways.

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Prostate Cancer Diagnosis and Therapy

Prostate Specific Antigen (PSA) tests, imaging studies, and prostate tissue biopsies are the mainstays of standard PCa diagnostic techniques (12). The efficacy of PSA testing is a subject of debate, owing to the occurrence of false positive results, which may lead to excess diagnosis and therapy of low-risk groups PCa with limited benefits (13). Ionizing radiation exposure during imaging tests can be expensive as well as harmful to health. Although CT scans are more expensive, they are known to have limited efficacy in identifying metastatic tumors or relapses of PCa in males with low levels of PSA. In the scientific field of therapeutics, the confinement of cancer to the prostate gland is classified as localized and has the potential for effective treatment. Radical prostatectomy, radiation, and surveillance are available as management options, however, there is little data to assess the merits of each strategy (14). Also, each approach has its own drawbacks, because cancer relapse is frequently observed in the case of targeted therapy and these methods are also intrusive, painful, and expensive.

PCa treatment options are currently limited to selective therapeutic drugs, including galeterone, abiraterone, and seviteronel, which are currently undergoing development. The present study evaluated the association and efficacy of Morusflavone flavonoid derived from Morus alba L., with CYP17A1. The FDA-approved CYP17A1 inhibitor functions by suppressing androgen production. CYP17A1 inhibition represents a significant therapeutic objective for the treatment of PCa. The results of a molecular dynamics simulation study suggest that morusflavone is a promising therapeutic target for PCa, since it is more stable than abiraterone and interacts with CYP17A1. There is a lack of data about the use of powerful naturally occurring anticancer chemicals like vinca alkaloids in the treatment of PCa (15).

Multi-Omic Approaches in Prostate Cancer Diagnosis and Management

The ineffectiveness of conventional approaches prompted researchers to come up with efficient and cutting-edge solutions to the present problems with PCa diagnosis and treatment. The advent of omics technology has led to unique initiatives aimed at characterizing the molecular alterations that underlie the onset and progression of various intricate medical conditions such as cancer (16). The field of cancer biology has increasingly relied on the acquisition and synthesis of information obtained from diverse sources, particularly with the advent of sequencing technology. One of the main difficulties related with the use of omics sciences in the diagnostic and therapeutic sectors is tumor heterogeneity, which makes it challenging to develop biomarkers that precisely reflect the characteristics of the entire tumor. Furthermore, data integration from multi-omics platforms is required for collecting and analyzing enough tissue samples.

Genomics, transcriptomics, proteomics, and metabolomics approaches can all be utilized today to thoroughly analyze the underlying mechanisms and to understand the numerous variations taking place (16). Particularly for advanced PCa, molecularly driven therapeutic targets are anticipated to enhance intervention as part of customized treatment plans based on novel, more targeted medicines, directed by omics-based biomarkers (17). Light has been shed on PCa etiology by genome-wide association studies, which have identified numerous predisposition loci and highlighted the importance of genetic variations (18). On the examination of PCa gene drivers, disease subgroups are identified, and therapeutic alternatives are created for precision medicine techniques.

Given the high correlation among the expression of many genes, the transcriptomics approach is commonly employed to assess the regulation of genes and to identify tumor subtypes (19). When mRNA profile of PCa were constructed, non-coding RNAs (ncRNAs) in the growth of cancer were discovered to be enhanced after radiotherapy, and the presence of this particular factor may indicate an adverse prognosis for the overall survival of individuals diagnosed with PCa (20).

Proteomics, being an omics approach, has been extensively employed in various research endeavors aimed at identifying biomarkers for PCa. This is due to its ability to directly reflect cellular activity and identify dysregulations in a variety of biological constituents (21). Proteomic alterations have been linked to metabolic activity, DNA repair, cell cycle regulation, and proteasomal degradation. Shina et al.'s study analyzed various Omics methodologies and assessed the precision of each biomarker. They discovered that proteomic characteristics were much more relevant than genomic, epigenomic, or transcriptomic features for predicting biochemical relapse (22). In a study conducted by Maria et al., the PNT1A, DU145, PC3, and LNCaP prostate cell lines were used to identify potential protein candidates associated with the progression of PCa (23). Tonry conducted a comprehensive assessment of the application of proteomics in the identification and personalized management of PCa (24).

Metabolomics has provided additional support in the characterization of the distinct metabolic profile associated with the progression of PCa and in the identification of metabolic alterations, which might be helpful as clinical biomarkers. To achieve this objective, several metabolomics studies have been conducted on PCa samples in recent times. Many technological advancements are currently accessible for the purpose of identifying and quantifying diverse metabolites in cells, tissues, or biofluids (25-27). Analytical procedures are based on mass spectrometry (MS) and nuclear magnetic resonance (NMR). The metabolomes from healthy and cancerous prostate tissues differ in lipid, nucleotide, Tricarboxylic acid (TCA) cycle, polyamine, and hexoamine production (28, 29). PCa is known to have elevated de novo lipogenesis (30), and cell lines produced from PCa metastases have upregulated levels of various lipid types. Urine metabolomics is a prompt and precise approach for the identification of diagnostic biomarkers for PCa, as well as predictive response biomarkers. The utilization of a metabolic signature has been proposed as a means of prognosticating diagnosis (31). According to independent research (25, 32), several metabolites—including a great number of those associated with the synthesis of energy, TCA cycle, and the metabolism of amino acids—are changed in urine. The omics approaches that are being employed in diagnostics and therapeutics of PCa are described in the following paragraphs.

Genetics of Prostate Cancer

Among all PCa risk factors, the patient's genetic makeup is considered the most significant one: according to reports, a person has 50% chances of developing PCa if an individual in their family has this disease (33). . To confirm the hereditary link of PCa, scientists have conducted many studies in which they have used twin, case-control, and family groups; the results showed that specific genetic mutations in people are increasing the risk of developing this disease (34). Different genes linked to PCa are listed in **Table 1**.

BRCA1, BRCA2, and ATM are the DNA repair genes, which are present in 5.5% of the men with PCa (35). Point mutations in the DNA sequences, such as single nucleotide polymorphisms and somatic copy number alterations, are relevant to the development of PCa because they silence the transcriptional activation of tumor suppressor genes, thus making the oncogenes functional (36, 37). The mutations during DNA replication in the nucleus pass on to the next generation, leading to the development of PCa due to the uncontrolled growth of cells with these mutations (38).

Genomics of Prostate Cancer

Almost all primary and metastatic PCa patients have been linked to mutations in the somatic genes (such as AR, WNT, PI3K-PTEN) and in the cell cycle signaling and DNA repair pathways. Different large genome studies have been conducted to find the association between metastatic castration-resistant (mCRPC) and PCa, which can be because of mutations in the genes, gene fusion, copy number variations of DNA, and rearrangements of genes (39, 40). In 1948, when cell free DNA (cfDNA, or the portion of circulating nucleic acid) was discovered in the blood (41). By conducting different research on PCa patients, scientists found out that, compared to healthy people, they had a higher number of longer cfDNA fragments, which increased concurrently with the stage and severity of the disease (42).

Transcriptomic of Prostate Cancer

The total number of RNA transcripts in an organism can be identified by transcriptomic studies. With the help of this, a total of 11 RNAs have been studied: among them, the mRNA, being translated into a protein after being transcribed from DNA, is the most concerned in cancer (43). The specific tumor type can be identified with the help of transcriptomic studies by measuring the expression of the genes: a higher gene expression means that they are closely related to each other and also are linked to tumor (19).

PCa progression can be predicted by the change in the mRNA level. This change will help in determining the difference between the normal and the metastatic state of PCa. Nine different stage-specific candidate genes linking to PCa progression are listed: *GSTP1*, *TP63*, *MYC*, *CENPA*, *EZH2*, *PIK3CB*, *HEATR5B*, *DDC*, and *GABPB1-AS1* (44, 45). The detailed transcriptome studies not only focus on the mRNA, but also include non-coding RNAs and their subtypes. The next generation sequencing (NGS) technique is used to study the transcriptomic profile of cells or tissues in detail (46-49).

The RNA biomarkers of PCa are listed in Table 2.

Table 1. List of genes linked to PCa and related syndromes.

Gene	OMIM of the Gene	Gene Location	PCa Histologic Characteristics	Inheritance	OMIM of the Pathology	Related Pathologies
MAD1L1	602686	7p22.3	PCa, somatic	.	176807	- Mosaic variegated aneuploidy syndrome 7, with inflammation and tumor predisposition; - Lymphoma, B-cell, somatic.
PTEN	602053	10q23.31	PCa, somatic	.	176807	- Macrocephaly/autism syndrome; - Cowden syndrome 1; - Meningioma Lhermitte-Duclos disease; - Glioma susceptibility 2.
KLF6	602053	10p15.2	PCa, somatic	.	176807	- Gastric cancer, somatic.
MXI1	600020	10q25.2	PCa, somatic	.	176807	- Neurofibrosarcoma, somatic.
BRCA2	600185	13q13.1	PCa	AD, SMu	176807	- Fanconi anemia, complementation group D1; - Glioblastoma 3; - Pancreatic cancer 2; - Breast cancer, male, susceptibility to; - Breast-ovarian cancer, familial, 2; - Medulloblastoma.
ZFHX3	104155	16q22.2-q22.3	PCa, somatic	.	176807	- Prostate cancer, somatic
CHEK2	604373	22q12.1	PCa, familial, s usceptibility to	AD, SMu	176807	- Li-Fraumeni syndrome 2; - Osteosarcoma, somatic; - Breast cancer, susceptibility to; - Colorectal cancer, susceptibility to.
AR	313700	Xq12	PCa, susceptibility to	AD, SMu	176807	- Androgen insensitivity; - Androgen insensitivity, partial, with or without breast cancer; - Hypospadias 1, X-linked; - Spinal and bulbar muscular atrophy of Kennedy.

Table 2. List of potential biomarkers for PCa, including long non-coding RNAs, circular RNAs, and microRNAs.

lncRNAs	Expression	Sample	Potential Biomarker	References
PCA3	Increased	Tissue/urine	Diagnostic/therapeutic	(50-54)
MALAT1	Increased	Tissue/plasma	Diagnostic/predictive	(55-59)
SChLAP1	Increased	Tissue/plasma/urine	Diagnostic/prognostic	(60-62)
FR0348383	Increased	Tissue/urine	Diagnostic	(63, 64)
PCAT1	Increased	Cell lines/tissues	Therapeutic	(65)
CCAT2	Increased	Tissues	Prognostic	(66)
CTBP1-AS	Increased	Tissues	Prognostic	(67)
DRAIC	Decreased	Cell lines	Prognostic	(68)
HCG11	Decreased	Tissues	Prognostic	(69)
LINC01296	Increased	Cell lines/tissues	Prognostic	(70)
LincRNA-p21	Decreased	Cell lines	Prognostic	(71)
LncRNA-ATB	Increased	Tissues	Prognostic	(72)
LOC440040	Increased	Cell lines/tissues	Prognostic	(73)
NEAT1	Increased	Cell lines/tissues	Prognostic	(74)
PCAT14	Increased (early)/ decreased (late)	Tissues	Prognostic	(75)
PCGEM1	Increased	Tissues	Prognostic	(76, 77)
TRPM2-AS	Increased	Tissues	Prognostic	(78)
UCA1	Increased	Tissues	Prognostic	(79)
circRNA				
circMYLK	Increased	Tissue	Diagnostic/therapeutic	(80)
miRNA				
miR-96	Increased	Tissue	.	(81)
miR-96-5p, miR-183-5p	Increased	Tissue	.	(82)
miR-145-5p, miR-221-5p	Decreased	Tissue	.	(82)
miR-221	Decreased	Tissue	.	(83)
miR-21, miR-22, miR-141	Increased	Plasma	.	(84)
miR-141, miR-375	Increased	Serum, tissue	.	(85)
miR-20a, miR-21, miR-145, miR-221	Increased	Plasma	.	(86)
miR-107, miR-574-3p	Increased	Urine	.	(87)
miR-200b, miR-200c	Increased	Plasma	.	(88)

Metabolomics

The primary objective of metabolic analysis is to quantify and characterize a maximum number of metabolites, with the ideal outcome being a comprehensive depiction of the metabolome. Biochemical pathway-related metabolic alterations can help uncover complex disease reasons. All of this information might lead to the identification of novel biomarkers for disease within current diagnostic procedures (89).

Proteomics and Biomarkers

According to recent investigations on cancer, only 10% to 20% of changes in proteome analyses may be attributed to changes in the transcriptome (90). Proteomics has been used in PCa biomarker research because it provides an instant analysis of the functioning of cells and reveals alterations in the most treatable biological components (21). By integrating the genomic data with the proteome of the tissue, it is possible to discover biomarkers and locate potential therapeutic targets. Furthermore, in situ histopathology permits researchers to further investigate the genetic basis of cancer initiation and progression. By using 2-Dimensional differential gel electrophoresis (2D-DGE) and western blotting, many protein indicators were identified as the PCa biomarkers, like UBE2N, Ser/tre-protein phosphatase PP1β (PPP1CB), and PSMB6 (91). SMARCA4 deletion impacts the chromatin accessibility and thus the gene regulation of a subset of AR genes, as well as CRPC development and dissemination (92).

Proteomic comparisons of PCa normal and cancerous tissue are also used to learn about the carcinogenic process. Interindividual differences can be ruled out by analyzing the prostate tissue with distinct histological patterns. PCa tumor stroma has more calcium-binding, intercellular interstitial, and smooth muscle contraction proteins than normal stroma (93). A significant contributor to the overtreatment of men with PCa is PSA, which is the best-known biomarker for PCa diagnosis and also a frequently employed biological indicator in investigating cancer (94).

Proteomics can identify biomarkers and therapeutic targets in health and disease systems biology. Precision medicine and proteomics help precision oncology in analyzing complicated carcinogenic pathways and targeted therapies, finding novel biomarkers for screening and detection, and evaluating therapy effectiveness and toxic effects (95).

Some of the major proteomics biomarkers responsible for renal cell carcinoma are mentioned in **Table 3**.

Table 3. List of Proteomics biomarkers linked to PCa.

Proteomics Biomarkers	Protein Family	Expression status	Assays for identification	References
PPP1CB	Metabolic proteins Plasma proteins	Decreased	2D-DGE MS	(91)
Ubiquitin-conjugating enzyme E2N	Cancer-related genes Enzymes Metabolic proteins Plasma proteins	Increased	2D-DGE MS	(91)
Coatomer protein complex, subunitα	Disease-related genes Metabolic proteins Plasma proteins	Increased	Immunohistochemistry (IHC) MS	(96)
Vinculin	Disease-related genes Plasma proteins	Increased	2D-DGE MS	(97)
Transthyretin	Cancer-related genes Human disease-related genes Plasma proteins	Increased	MALDI-TOF MS, MS, 2D-DGE, IHC	(98)
MethylcrotonoylCoenzyme A carboxylase 2 (beta)	Disease-related genes Enzymes Human disease-related genes Metabolic proteins Potential drug targets	Increased	MALDI-TOF MS 2D-DGE, IHC, Western Blotting	(99)
Periostin	Cancer-related genes Plasma proteins	Increased	2D LC-MS/MS and iTRAQ	(100)

Lipid omics and biomarkers

Disease research recently adopted lipidomics. The identification of different lipid biomarkers for particular health issues is important, because many diseases cause unique and distinctive alterations in the lipid compounds of bodily fluids or tissues before clinical symptoms appear (101). The vast array of lipids presents a significant challenge in the research and development of analytical techniques for lipidomics. MS, particularly in conjunction with chromatographic separation methods, is a highly prevalent approach in the field of lipidomics. The quantitative examination of lipids in biologic specimens using MS has yielded copious data that can be used for the clinical assessments of various diseases (102). The implementation of the lipidomics approach has gained significant traction in cancer research due to its ability to accurately delineate the lipid structures and compositions present in specific cells or organisms (103). Different mitogens—such as lysophospholipds, lysophosphatidic acids, phospholipids, and phosphatidic—are responsible for PCa. Lipid kinases, G protein-coupled receptors, and small G proteins are major factors responsible for the complications in different cellular signaling pathways and cytoskeletal rearrangements (104).

In the case of lipidomics, a lipid profile and metabolic pathway can be constructed. This procedure involves the extraction of lipids from tissues and cells, followed by lipid analysis, which eventually contributes to the construction of the lipid profile, as well as its analysis and subsequent pathway analysis (103). The study conducted by Zhou X. et al. aimed to explore the potential diagnostic and prognostic significance of lysophosphatidylcholine transferase 1 (LPCAT1) in prostate tumors by using IHC on tissue microarray slides. The study examined the association between LPCAT1 expression and cancer advancement. The pivotal function of LPCAT1 in the modification of PLs and its upregulation in various carcinomas (such as colorectal and prostate) in contrast to healthy mucosa had previously been established (105, 106).

Microbiomics and Biomarkers

Current research indicates that changes in the composition of microbiota, known as dysbiosis, may have a significant impact on the onset, progression, and outlook of PCa. The microbiome, which encompasses the entirety of microorganisms and their genetic material residing on and within the body, is acknowledged as a significant factor in the identification of various cancer types. Various extensively researched human microbiomes, consisting primarily of diverse bacterial populations, possess the capacity to act as etiological factors in carcinogenesis and/or influence the individual's response to therapeutic interventions (107). There are limitations in case of microbiomics biomarkers for PCa specifically with PSA but still some of the biomarkers with increased expressions involved in the progression of PCa. Potential prognostic and diagnostic biomarkers for PCa are human endogenous retrovirus, herpes simplex virus derived HSV2-miR-H9-5p and HSV1-miR-H18 (108-111). No data exists on how microorganisms affect therapy response. Further investigation is required to explore the correlation between dysbiosis of the gastrointestinal tract and genitourinary microbiome, persistent inflammation and the development of PCa. Results could help develop innovative approaches and risk stratification methods (107). Some of the major microbiomics biomarkers associated with PCa are listed in table 4.

Table 4. Microbiome biomarkers associated with PCa.

Biomarker	Characteristics	Family	Stimulus	Molecular Targets	Role in carcinogenesis	References
E. coli	Gram-negative and rod-shaped	Enterobacteriaceae	Cytotoxic necrotizing factor 1 and Lipopolysaccharide	Toll-Like Receptor (TLR), CDC42 and Nuclear Factor kappa B (NF-Kb)	Avoidance of apoptosis, promoting inflammation and metastasis	(112)
Staphylococcus	Gram-positive and sphere-shaped	Staphylococcaceae	Staphylococcal enterotoxin	lncRNAs	Activation of immune system	(112)
Human papillomavirus	DNA virus	Papillomaviridae	E2, E6, and E7 (Enveloped proteins)	Tumor Necrosis Factor Alpha (TNF-α), Vascular Endothelial Growth Factor (VEGF), Interleukin 6 (IL-6), Reactive oxygen species (ROS), and NF-κB	Proliferation and survival of cancer cells	(112)
Adenovirus	Double-stranded DNA and enveloped	Adenoviridae	Fas ligand (FasL)	FasL-mediated apoptosis	Progression of cancer cells	(113)
Chlamydia trachomatis	Gram-negative	Chlamydiaceae	-	NF-κB, IL-6, TLR2-4 and FGF-2	Metastasis Vascularization	(114)

Future directions

Personalized medicine can facilitate the development of treatment strategies using omics. The early detection of PCa can prove to be a viable strategy, and additional investigations may yield more effective therapeutic interventions. Various omics technologies can aid in understanding the heterogeneity of tumor microenvironment of specific cancer types, thus helping in the development of a treatment. The current state of the diagnostic test does not permit its application in a clinical setting; further investigation is required to authenticate biomarkers, ascertain their therapeutic viability, and incorporate appropriate protocols. Miniaturized assays and multiplexing technology have the potential to facilitate the development of biomarker tests.

Conclusion

In this age of big data, researchers are using omics technologies like metabolomics, transcriptomics, and genomics to search for diagnostic markers in a wide range of diseases. Diagnostic research and disease surveillance in humans and economically relevant animals are two areas in which omics data are rapidly becoming crucial. This new era in clinical care calls for cutting-edge approaches, and lipidomics has been considered as one of the most promising. Health and economic benefits of the omics test should be established through prospective trials, and the test should be made more accessible to patients. Different novel targets and biomarkers can be identified for clinical applications by studying the oncometabolite and its association with different signaling pathways. The use of omics data has led to an improvement in the precision and dependability of diagnostic and prognostic assessments. Targeted therapy, when efficiently executed, has the potential to minimize the toxic effects on normal cells in comparison to chemotherapy.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Omics sciences and precision medicine in breast and ovarian cancer

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Abstract

Background: Human breast carcinoma is a complex disease, affecting 1 in 8 women worldwide. The seriousness of the disease increases when the definite cause of the disease remains obscure, thus making prognosis challenging. Researchers are emphasizing on adapting more advanced and targeted therapeutic approaches to address the multifaceted impacts of the disease. Hence, modern multi-omics systems have gained popularity among clinicians, as they offer insights into the genomic, pharmacogenomic, metabolomic, and micro-biomic factors, thus allowing researchers to develop targeted and personalized approaches for breast cancer prevention and early detection, and eventually improving patient outcomes.

Aim. The primary focus of this study is to elucidate, through the integration of multi-omics research findings, the inherent molecular origins of diverse subtypes of breast cancer and to evaluate the effectiveness of these findings in reducing breast cancer-related mortalities.

Methods. Thorough investigation was conducted by reviewing reputable and authoritative medical journals, e-books, and online databases dedicated to cancer research. The Mendelian inheritance in man database (OMIM) was used to scrutinize specific genes and their respective loci associated with the development of different types of breast cancer.

Results. Our present research revealed the holistic picture of sundry molecular, genomic, pharmacogenomic, metabolomic, and micro-biomic features of breast cancer. Such findings, like genetic alterations in highly penetrant genes, plus metabolomic and micro-biomic signatures of breast cancer, unveil valuable insights and show great potential for multi-omics research in breast oncology.

Conclusion. Further research in omics sciences pertaining to breast cancer are at the forefront of shaping precise treatment and bolstering patient survival. ***Clin Ter 2023; 174 Suppl. 2 (6):104-118 doi: 10.7417/CT.2023.2477***

Key words: *breast cancer, precision medicine, genomics, metabolomics, cancer metabolism, biomarker*

Introduction

Breast cancer (BC) has turned out to be one of the most insidious female malignancies worldwide. It is responsible for substantial morbidity and mortality, posing a huge burden on healthcare systems internationally. Recently, GLOBOCAN report (2020) estimated about 2.3 million new breast cancer incidences in a year, which led to 684,996 cancer-related mortalities (about 6.6%) globally (1-3). BC has ranked second among the most frequently diagnosed malignancies, comprising over 11.6% of all female cancers, and placed fifth amongst the most prevalent causes of cancer fatalities (2-4).

Breast cancer is a complex disease that exhibits vast histologic, genetic, and molecular diversity. Even though it is spread worldwide, its occurrence, fatality, and life

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expectancy differ greatly in the different areas of the world because of the variable lifestyles of different populations and their genetics, which are known to cause this malady (5). Evidence suggests that BC occurrence is higher in developed countries than in the developing ones (2, 6). However, it has been noted that the rate of BC incidences in high-income countries has met a stabilization and decline, probably due to medical advancement, while its rate in low-income countries has increased (1,3).

BC is most commonly observed in women aged 40-64 years (1, 4). The lifetime risk of females developing breast cancer is approximately 1 in 9 women, with a five-year survival rate as low as <30% (6). Moreover, BC also develops in males, accounting for <1% of all male cancers. This rare cancer type in males is often underestimated and might remain undiagnosed at its early stages because of negligence in checking BC-related risk factors, which are same as female breast cancer: some examples are old age, hormonal dysregulation, radiation exposure, and mutations in BRCA genes (7, 8). Diagnosis is usually made either through screening methods (e.g., X-ray mammography, ultrasonography, etc.), or by detecting particular BC biomarkers at a molecular level, through techniques like immunohistochemistry (IHC), real-time PCR, and nucleic acid hybridization system (9).

Among BC cases, 10% are due to genetic predisposition, while other risk factors include age, environment, obesity, unhealthy diet, use of alcohols & contraceptives, and hormone replacement therapy; the majority of which are potentially modifiable (4, 5). Most susceptible genes responsible for causing breast cancer (BC) also cause ovarian cancer (OC), which commonly leads to hereditary breast-ovarian cancer (HOBC) syndromes (10). BRCA1 and BRCA2 genes have been identified as the mostly examined pathogenic variant genes associated with high risk of breast and ovarian cancer (11). However, there are some non-BRCA genes that are known to increase BC and OC risk (10).

Modern technological advancements in the field of 'omics' studies have undoubtedly helped a lot in clarifying genetic etiology and pathological changes in breast cancer. An extensive availability of multi-omics databases to pile up large-scale genomic, epigenetic, transcriptomic, and proteomic information is crucial for patient stratification, early prognostication of biomarkers and development of potentially targetable precision treatment to upgrade overall survival (12, 13).

Review articles in cancer research provide a comprehensive overview of numerous researches, also aiding in therapeutic planning and clinical management (5). In this review article, we intended to delve deep into the oncology of breast cancer in the context of its genetics, genomics, pharmacogenomics, metabolomics, and micro-biomics research, to elaborate genomic and histopathologic characteristics of BC for the development of meticulous prognosis and therapeutic management in the future.

Histopathologic findings and BC classification

Breast cancers exhibit immense heterogeneity in histopathological features and oncogenesis, posing great challenges for diagnosis and clinical decision making. Histologically, BC can be identified by uncontrolled cell growth either in the ducts or in the lobules of breasts. Depending on its anatomical origin, breast carcinoma broadly falls into two categories:

1. Invasive ductal carcinoma (IDC): the most common type of BC (accounting for about 80% of all cases). It begins in the cells that line the milk ducts and grows into the surrounding breast tissues (14);
2. Invasive lobular carcinoma (ILC): the less common type of BC (accounting for about 10-15% of all cases). It begins in the cells of milk-producing glands, or lobules (14).

In addition, the classification of invasive BC is also done through the 3-tier (low grade, intermediate grade, and high-grade) system, which comprehends the microscopic appearance of tumors (12). However, the extent and severity of BC is determined by a definite staging system, which is a different concept from grading. Staging refers to the process of determining the tumor potential to metastasize in stages from 0-IV. It is based on anatomic findings like the percentage of tumor (T) in breast tissues, the degree of lymph nodes involvement (N), and mitotic rate or metastasis (M). Both systems are heavily utilized in clinical practice and important to determine the best course of treatment (12, 14). A widely used method of breast cancer staging is the Nottingham Prognostic Index (NPI), that combines the scores of different histologic and molecular features to state the prognosis (3, 14).

Molecular features and subtyping of BC

An updated prognostic system of breast cancer staging has been published by the American Joint Committee on Cancers (AJCC) 8th edition in 2018, which also acknowledges molecular features of BC in addition to histological features (3).

On the basis of mRNA gene expression levels, BC has been divided into four intrinsic or molecular subtypes, named as luminal A, luminal B, HER2-enriched, and basal-like or triple-negative BC (3, 12). The additional features included in this classification are:

- Estrogen receptor (ER) expression levels,
- Progesterone receptor (PR) expression, and
- Oncogenic Human Epidermal growth factor Receptor-2 (HER2) overexpression (12).

It is worth mentioning that ER and PR are the receptors that cause the stimulation of cellular growth in normal and neoplastic conditions, and they are overexpressed in nearly 75% of BC cases. HER2 is found overexpressed in 15% of BC cases, and is characterized by aggressive invasion and poor prognosis. ER, PR, and HER2 overexpression serves as an important biomarker and is predictive of hormonal and anti-HER2 targeted therapy. Sometimes, about 10-15% of BCs are not diagnosed with either of three biomarkers and are thus called triple negative breast cancers (TNBC) (12).

This molecular subtyping of BC is now performed through the PAM50 gene expression test, which examines the activity of 50 different signature genes (15). It is able to classify BC into the abovementioned intrinsic subtypes with >90% accuracy. PAM50 uses a technology called quantita-

tive reverse transcription polymerase chain reaction (qRT-PCR) to measure the expression levels of these genes in a tumor sample, to help in the prognosis and guide treatment decisions in BC patients (3, 15).

Genetic basis of breast cancer

About 5-20% cases of breast and ovarian cancers are due to hereditary defects in pathogenic variant genes (11, 16). Studying genetics of breast cancer is thus essential to gain a better understanding of the genetic changes that lead to this malignancy, and to get help in designing targeted therapies and improving early detection of BC in high-risk individuals.

BRCA1 and BRCA2 are two well-known pathogenic variant genes predisposing individuals to breast-ovarian cancers. There are also certain non-BRCA genes and other hereditary predisposition syndromes leading to breast-ovarian cancers. Some well-recognized inherited syndromes include Lynch syndrome, NTHL1 tumor syndrome, MUTYH-associated polyposis, familial adenomatous polyposis, and hereditary breast and ovarian syndromes. Detecting these hereditary syndromes through genetic testing can be helpful in the identification of high-risk individuals, early discovery of breast and ovarian cancer probability, and timely personalized treatment (11).

Genetic testing generates a great quantity of data and its execution requires genetic counseling. Therefore, some mathematical models have been generated, which can predict the probability of carrying pathogenic variant genes and the breast-ovarian cancer risks by using the description of family history. One of such susceptibility models is BOADICEA (Breast and Ovarian Analysis of Disease Incidence and Carrier Estimation Algorithm), formulated by Antoniou et al. in 2004. This model predicts the susceptibility to breast cancer by combining the multiplicative effect of mutations in BRCA1/2 genes along with multiple other genes. Risk predictions by this model are nearly the same as those observed through population-based studies (17).

Another similar approach is the Mendelian Randomization (MR) model, which is a statistical technique used to investigate the causal relationship between a specific risk factor and the disease or health outcome. Based on Mendelian genetics, MR has been also used to investigate the risk factors of breast cancer. For instance, a study by Guo et al. used the MR tool to identify the causal relationship between body mass index and breast cancer risk. Genetic variants associated with BMI were examined as instrumental variables to estimate the causal effects. The study found that a higher BMI was causally associated with an increased risk of breast cancer (18).

One more similar study conducted by Vanhevel et al., utilized the MR approach to investigate the causal relationship between vitamin D levels and BC risk. The study used certain genetic variants responsible for vitamin D levels to estimate the causal effect of vitamin D on breast cancer risk. Results of the study showed that higher genetically predic-

Table 1. Selected inherited genetic alterations with genes and genetic syndromes that cause breast and ovarian cancers (3, 16, 20).

Inherited syndromes	MIMs of syndrome phenotypes	Genes	Cytogenetic locations	Gene OMIMs	Lifetime cancer risk	Inheritance
Hereditary Breast-ovarian cancer (HBOC) syndrome, familial, 1	604370	BRCA1	17q21.31	113705	Breast, ovarian, prostate	AD
Hereditary Breast-ovarian cancer (HBOC) syndrome, familial, 2	612555	BRCA2	13q13.1	600185	Breast, ovarian, prostate, melanoma, pancreatic, gall bladder	AD
Fanconi anemia, complementation group J	609054	BRIP1	17q23.2	605882	Ovarian	AR
Hereditary Diffuse Gastric and lobular breast Cancer syndrome	137215	CDH1	16q22.1	192090	Gastric, Lobular breast	AD
N/A	Nil	CHEK2	22q12.1	604373	Breast	AR
Fanconi anemia, complementation group N	610832	PALB2	16p12.2	610355	Breast, ovarian	AR
Cowden syndrome, Lhermitte-Duclos disease	158350	PTEN	10q23.31	601728	Breast	AD
BROVCA3	613399	RAD51C	17q22	602774	Ovarian	
BROVCA4	614291	RAD51D	17q12	602954	Ovarian	
Li-Fraumeni syndrome	151623	TP53	17p13.1	191170	Breast, soft tissue sarcoma, brain, osteosarcoma	AD
Lynch syndrome 4	614337	PMS2	7p22.1	600259	Ovarian	

Abbreviations: *AD, autosomal dominant; AR, autosomal recessive; MIM, mendelian inheritance in man, BROVCA, breast-ovarian cancer.*

ted vitamin D levels possess anti-cancer activity and were associated with a lower risk of breast cancer (19). In short, MR has the potential to provide valuable insights into the underlying causes of breast cancer and help in the development of new prevention and treatment strategies.

It has been proved that about 5-20% breast and ovarian carcinomas are due to genetic abnormalities. Researchers have attempted to explore the exact polygenic genetic architecture responsible to cause BC: in 2019, Lu and colleagues obtained a set of 11 susceptibility genes potentially responsible to cause BC and OV through large-scale exome sequencing (16). Here, we are enlisting these predisposition genes (**Table 1**) with MIM (Mendelian inheritance in man) status, whose variants are responsible for causing breast cancers.

Multigene panel testing identifies pathogenic variants that harbor the risk of breast and ovarian cancers (16). BRCA1, BRCA2, TP53, PTEN, STK11, and CDH1 are the genes that are considered as highly penetrant genes that predispose to breast-ovarian cancer, while PALB2, BRIP1, CHEK2, and other Fanconi anemia genes are considered as low or moderate penetrant genes that predispose to breast and ovarian cancers. The following paragraphs will give a brief description of some predisposition syndromes that increase BC risks (21).

Hereditary breast and ovarian syndromes

BRCA1 and BRCA2 are high penetrance genes that are known to cause hereditary breast-ovarian cancer syndromes (BROVCA). The gene BRCA1 is located on 17q21.31, that codes for BRCA1 protein. It is a tumor suppressor protein that acts in combination with DNA damage sensors and signal transducers to make a huge protein complex called BRCA1-associated genome surveillance complex, or BASC (21). On the other hand, BRCA2 is located on chromosome 13q13.1 and codes for a nuclear protein responsible for DNA repair by homologous recombination. Mutations in BRCA1/2 lead to the high risk of breast-ovarian cancer in females, along with increased risk of developing BC in males (21).

Pathogenic variants of a single BRCA gene inherited from one of the parents do not always lead to the development of BC, but a second mutation that could affect the pathogenic variant may increase the susceptibility of BC. Therefore, BRCA1/2 mutations possess great prognostic value and remain the focus of genetic testing to predict the risk of BC (14).

Lynch syndrome

Lynch syndrome (LYNCH), also called hereditary non-polyposis colorectal cancer, is an inherited condition that increases the risk of developing several cancers, including ovarian cancer (22). It is an autosomal dominant disorder that can be diagnosed through early onset of colorectal cancer. Lynch syndrome is caused by mutations in the PMS2 gene, located on chromosome 7p22.1, which is a DNA mismatch repair gene. Multiple studies have observed the presentation of colorectal cancers due to monoallelic mutations in PMS2 (23).

NTHL1 tumor syndrome

NTHL1 tumor syndrome refers to a genetic condition, caused by mutation in the NTHL1 gene located on chromosome 16p13.3, responsible for code repair enzymes like DNA N-glycosylases of the endonuclease III family (24). NTHL1 is a DNA repair gene, and its mutation may favor the growth of various cancers, including breast cancers. This inherited condition is also called familial adenomatous polyposis (FAP), and is associated, although rare, with an increased risk of breast cancer (25).

MUTYH-associated polyposis

MUTYH-associated polyposis (MAP) is an inherited syndrome, characterized by multiple polyps in the colon. MUTYH is a base excision repair (BER) gene, located on chromosome 1p34.1, responsible to sidestep DNA damage from methylation, deamination, reactive oxygen species, and hydroxylation. Germline biallelic MUTYH pathogenic variants are correlated with the development of MAP, which markedly increases the chances of colorectal cancer (CRC) development. In addition, the risk for malignancies of the bladder, breast, ovary, and endometrium also increase (26). Identification of MUTYH mutations by genetic testing can predict the risk of suspected cancers and can improve surveillance and intervention strategies to counteract cancer incidences in the future (11).

Li-Fraumeni syndrome

Li-Fraumeni syndrome is an autosomal dominant disorder caused by mutation in TP53 gene, located on chromosome 17p13.1. It encodes for TP53 protein, which is an important tumor suppressor protein that induces cell cycle arrest, apoptosis, and DNA repair mechanisms. TP53 mutation causes great predisposition to various cancers such as brain, sarcoma, and breast cancer. Therefore, due to increased chances of developing BC, individuals with Li-Fraumeni syndrome are suggested to keep regular examination for BC every 6-12 months, with MRI screening annually (21).

Genomic hallmarks of Breast Cancer

Breast carcinogenesis emerges by inherited genetic variations and acquired genomic aberrations that lead to abnormal cellular proliferation. Mutations of specific genes make up the molecular basis of any cancer, however, malignant transformation is a multi-step process that also depends on somatic mutations, copy number aberrations, DNA repair defects, epigenetic changes, and structural rearrangements of chromosomes like deletions, amplifications, inversions, and translocations. Delineating these events is only possible by whole genome sequencing, which is becoming a standard research tool to study breast cancer progression (27). Genomics is a powerful approach to analyze family history of breast cancer in high-risk individuals, who thus may be subjected to prior screening and will be benefitted with early diagnosis and risk assessment of the disease (3,28).

Correlated pathologies, as lymphedema, can also have a genetic cause (29-34)

Whole genome profiling of large cohorts of breast cancer has demonstrated different deviational processes that are distinct in each subtype, termed as genomic mutational signatures (35). These mutational processes include abnormal DNA editing, aberrant replication, base substitution, tandem duplication/deletion, flawed DNA repair, mismatch repair deficiency, and mutations by carcinogen exposures (12). Each particular BC subtype may harbor more than 1 mutational signature, and thus aid in patient stratification.

Genomic hallmarks of breast cancer are crucial to attain a more holistic picture of BC complexities. For this purpose, The Cancer Genome Atlas (TCGA) has used six different domains of information that provide the basis of genomic instability in BC (12). Here we will briefly discuss each domain, to discover breast cancer's genomic diversity.

1. **Distinct mRNA expression of BC subtypes-** mRNA expression microarray is a powerful approach, used to measure the expression levels of thousands of genes simultaneously in a breast cancer tissue. This approach has been used to identify the genes that are differentially expressed in cancerous and normal breast tissues, and to spot the genes responsible for cancer development and progression. Depending upon mRNA expression, BC has been classified into 4 intrinsic subtypes, named luminal A and B, HER2 positive, and basal-like or triple-negative BC (36).
   Each BC subtype showed signature mutations: for example, luminal A and B exhibited mutations in PIK3CA, GATA3 or MAP3K1 genes, while triple-negative BC showed mutations in BRCA1, TP53, and RB1 genes with MYC amplifications (35). This subtyping is very helpful in predicting patient outcomes and their response to specific treatments like hormone therapy or chemotherapy (36).
2. **Differential DNA methylation of specific subtypes**- DNA methylation signatures in BC have valuable diagnostic potential and comprise a robust system to improve disease management. DNA methylome sequencing is performed through a technique called DNA methylation chips, through which we can measure the methylation status of numerous CpG sites across the genome (12).
   DNA methylation is an essential epigenetic mechanism, in which a methyl group is added to the cytosine nucleotide of a CpG dinucleotide. This mechanism is important to regulate gene expression, and its aberration causes gene expression abnormality and cancer progression. For instance, the profiling of a methylome in triple-negative breast cancer showed that differentially methylated regions (DMRs), associated with TNBC, serve as potential biomarkers for this BC subtype (37). Moreover, the association of specific DMRs with different BC subtypes has implications for diagnosis and therapeutic outcomes.
3. **Single nucleotide polymorphism (SNPs)-** SNPs in BC may be germline (inherited) or somatic (acquired) mutations that have potential to improve precision of BC risk prediction. BRCA1/BRCA2 are the most commonly observed germline mutations, which are known as BC-predisposition pathogenic variants. Next generation DNA sequencing has also led to the discovery of additional germline mutations—including BARD1, PALB2, RAD51D, BRIP1, RAD51C, and TP53—that are linked with moderate to high risk of triple-negative BC (38). Currently, the Genome Wide Association Studies (GWAS) database of published SNP-trait associations has identified 182 SNPs linked with BC, which are mentioned in about 53 studies (38).
   Somatic mutations potentially promote BC growth and metastasis, and can be specifically demonstrated in particular subtypes. Notably, a study by TCGA showed that three somatic mutations (TP53, PIK3CA, and GATA3) are common in all BC subtypes, with over 10% occurrence. Whereas, many subtype-associated mutations also exist in GATA3, PIK3CA and MAP3K1 in Luminal A BC (39).
4. **Aberration in microRNA (miRNA) expression-** miRNA are small non-coding RNAs that participate in post-transcriptional gene regulation. Their aberrant expression is known to promote cancer instigation, and it is also associated with breast cancer progression (12, 39). Moreover, the dysregulation of miRNA expression also shows distinct signatures that are used to classify BC subtypes and predict clinical outcomes. For example, a study showed that the downregulation of four miRNAs (miR-221, miR-1305, miR-4708, and RMDN2) in TNBC leads to more aggressive breast tumors, with poor prognosis (40). Similarly, the Luminal A subtype is further divided into two subgroups, depending on the differential expression of miRNAs (12).
5. **Significantly mutated genes (SMGs) and altered DNA copy number (CNAs)-** Whole exome sequencing of plenteous breast tissue samples showed 30,626 somatic mutations, including ample point mutations, few dinucleotide mutations, and abundant insertions/deletions (indels). This greatly helps in identifying frequently mutated genes in breast cancer—including TP53, PIK3CA, GATA3, and MAP3K1—and also driver mutations that promote BC growth and progression (39).
   Mutations like deletions, amplifications, and rearrangements cause changes in the number of copies of DNA segments in the genome, also called copy number alterations (CNAs). Large-scale studies have identified specific CNAs that are associated with breast cancer development and progression. Focal amplification of segments holding PIK3CA, EGFR, FOXA1, and HER2, and focal deletions of segments holding MLL3, PTEN, RB1, and MAP2K4 were found to cause CNAs in breast cancer (39).
   Similarly, a study analyzed genome rearrangements in breast cancer and their association with patient survival. In a large cohort of BC patients, genome-wide copy number alterations were analyzed to

discover certain novel and recurrent CNAs specific to BC subtypes. This copy number profiling has implications for making clinical decisions and targeting specific genome rearrangements in breast cancer (41).

6. **Presence of tumor antigens as protein biomarkers-** Reverse-phase protein array (RPPA) is an antibody-based technique that measures protein expression levels in BC tissues to identify protein biomarkers and drug targets, to provide insights into the molecular mechanisms underlying the disease, and to guide the development of personalized therapies. Protein microarrays have the potential to simultaneously present and assess hundreds of tumor antigens (42). A study used RPPA to screen for autoantibodies in serum samples from BC patients and healthy controls. The results of this study showed high sensitivity and specificity for the detection of early-stage breast cancer (42).

Similarly, on the basis of protein expression, two novel protein-defined subgroups of breast cancer were also made. These are reactive I and reactive II groups, composed primarily of a subset of Luminal A tumors and a mixture of mRNA subtypes. These groups are termed 'active' because of the occurrence of proteins generated in the tumor microenvironment and cancer-activated fibroblasts (39).

An expansion in genomics of the research on breast cancer is inevitable to gain the wide-ranging list of recurrent mutations found in BC. By sequencing tumor genomes at both early and advancing stages, we can pinpoint essential cellular pathways to annotate with pharmacogenomics and to identify potential therapeutic targets (27).

Pharmacogenomics of breast cancer

Selecting a particular drug for a breast cancer patient depends, to some extent, on their genetic makeup: genetic alterations, even of a single nucleotide, noticeably impact the activity and expression of proteins involved in the pharmacokinetics and pharmacodynamics of therapeutic drugs. Therefore, variations in germline DNA are the prime focus of pharmacogenomics, because they can significantly alter drug metabolism and its therapeutic outcomes (43). Ultimately, pharmacogenomic studies are aimed at identifying single nucleotide polymorphisms (SNPs) and other genomic alterations in particular patients, determining targets for selected drugs, and improving their efficacy and safety in clinical settings (43, 44).

The selection of suitable treatment for operable breast cancer involves both local therapies (surgery and radiation) and systemic therapies (by various drugs). Systemic therapy plays a crucial role in upsurging disease-free survival (DFS) by diminishing the tumor potential and controlling micro-metastasis (45). The drugs used for systemic therapy generally fall into three classes: hormonal therapy, targeted therapy, and chemotherapy. These therapeutic drugs can be given alone or in multi-drug regimens. In this review, we will be focusing on pharmacogenomics of drugs employed for breast cancer patients and their clinical applications with probable outcomes.

Stratification of patients for appropriate treatment

Phenotypic subtypes of BC are very important in selecting suitable treatment options (45). For example (44, 46, 47):

- Luminal A- ER/PR +ve – treated with endocrine receptors modulators like tamoxifen or aromatase inhibitors;
- Luminal B- ER/PR +ve – also treated with hormonal therapy;
- Metastatic Luminal BC – treated with selective CDK4/6 inhibitors such as Palbociclib;
- HER2 and TNBC – treated with Antibody–Drug Conjugates (ADCs);
- HER2 enriched- HER2 +ve – treated with anti-HER2 therapies, like trastuzumab;
- TNBC- All receptors negative – treated with chemotherapy.

Principally, the choice of suitable therapeutic strategy for an individual patient is a multi-disciplinary process, and many factors are to be considered. Various factors include age, menopausal status, physical and mental health, and clinical phenotypes such as tumor size, nodal status, invasiveness, expression of hormonal receptors, and the patient's genetic constitution (48). Decisions about systemic therapy are made by predicting drug responses and examining tumor sensitivity to drugs. Also benefits, adverse events, and costs are to be determined. For the decision of timings of systemic therapies, adjuvant (after surgery) and neoadjuvant (before surgery) methods intended to improve DFS are being tailored for each particular patient (45).

Like other cancer patients, BC patients possess two types of genomes. One is their own integral germline genome, and the other is the altered tumor somatic genome (44). The somatic genome is eccentric from their constitutional genome because it harbors inherited genetic alterations in addition to acquired genomic variations, which are either oncogenic 'driver mutations' or established during carcinogenesis as 'passenger mutations'. Somatic or driver mutations surmount principal focus in the quest of targeted therapy, because they are actively involved in tumorigenesis and provide a selective growth advantage to BC. On the other hand, passenger mutations do not confer much growth advantage to breast tumor and gained less attention of researchers because they are not ideal targets to develop precision drugs (35, 44).

These idiosyncratic genomic variations, which are responsible to cause pathogenic event or are influenced by drugs, are designated as genetic biomarkers (44). Genetic alteration of even one nucleotide can result in either lack or deviant enzyme activity, which produces a huge impact on drug response. This way, a single nucleotide polymorphism (SNP) can influence drug toxicity and efficacy in numerous ways (43).

Hormonal therapy

Nearly 80% of BCs are classified as estrogen receptor (ER)-positive, out of which 65% are also progesterone receptor (PR)-positive (43, 49). These receptors are actually

nuclear proteins that regulate the expression of specific genes and predict the patient's sensitivity to endocrine manipulations. Estrogen regulates various cellular activities, including breast tumor cell proliferation. Therefore, hormonal therapy is considered as complementary to surgery in the majority of cases, because it blocks hormonal receptors and inhibits tumor progression.

There are two major types of hormone receptor blockers that are approved for BC treatment, i.e., selective estrogen receptor modulators (SERMs), and the third-generation aromatase inhibitors (AIs). Since estrogen is the major culprit of causing BC in ER +ve patients, estrogen therapy (ET) can have different targets, which are mainly:

- Suppression of the ovaries' function, since they are the main source of estrogen and progesterone hormones;
- Use of selective estrogen receptor modulators (SERMs), which prevent the uptake of estrogen by the receptor;
- Use of selective estrogen receptor downregulators (SERDs), which, like SERMs, impede ER binding to cancer cells, thus reducing their proliferation and survival;
- Inhibition of the key enzyme involved in estrogen biosynthesis (aromatase inhibitors) (49).

All of the above stratagems have different pharmacologic properties, biochemical nature, and molecular organization, but ultimately they all disrupt estrogen signaling. Below we discuss each class of drugs that inhibit proliferation of estrogen-dependent BC cells, but taking different metabolism routes, which is controlled genetically (43).

Ovarian functional suppression (OFS)

Ovarian functional suppression (OFS) is usually achieved either through oophorectomy (surgical removal of one or both ovaries) or by infusion of gonadotropin releasing hormone agonists (GnRHa) (49).

Gonadotropin releasing hormone (GnRH) is an essential hormone for the synthesis of sex hormones. Released from hypothalamus, GnRH stimulates pituitary gland to produce luteinizing hormone (LH) and follicle stimulating hormone (FSH), the two of which cause the gonads to make sex hormones, i.e., testosterone in males, and estrogen and progesterone in females. GnRH agonists have been used to inhibit the levels of sex hormones because, after an initial transient surge in sex hormone levels, they cause a decline in their release. Luckily, GnRH analogs have proved to be much more effective and protracted in producing the same efficacy, without the initial surge. An excellent example of a GnRH analog is degarelix (50).

Selective estrogen receptor modulators (SERMs)

Selective estrogen receptor modulators (SERMs) are nonsteroidal molecules that can bind to ER and exert agonist or antagonist actions. Among them, the most effective and widely used SERM is tamoxifen (TAM), which is considered as standard adjuvant treatment prescribed at initial stages of BC to women over 30 years of age. Its use for five years substantially decreases the risk of recurrence and mortality by BC. Tamoxifen undergoes extensive metabolism in liver through cytochrome p450 enzyme system (CYP450), leading to the production of highly active metabolites that are more dynamic than its original form and show remarkable pharmacological impressions (43).

One of such metabolites is 4-hydroxy tamoxifen, which possesses thirty to hundred-fold greater potency in crushing ER-dependent cell proliferation than tamoxifen Similarly, endoxifen is another metabolite that also effectively inhibits ER activity and ER-positive cell proliferation. Genetic variations in genes that encode enzymes for tamoxifen metabolism have pharmacogenomic importance in predicting BC outcomes (43).

Selective estrogen receptor downregulators (SERDs)

Selective estrogen receptor downregulators (SERDs) are compounds capable of reducing the ER protein level and blocking ER activity, thus also acting as ER antagonists. A well-known ER downregulator is fulvestrant, which binds with ER, thus preventing its dimerization and eventually leading to its degradation.

Fulvestrant is an FDA-approved SERD for BC patients that has greater affinity for ER and causes less adverse effects on endometrial ERs. Particularly, it is useful for patients with advanced BC stages and serves as second-line therapy in TAM-resistant BC patients (49,51).

Aromatase inhibitors (AIs)

Third-generation aromatase inhibitors work by counteracting the key enzyme aromatase, which converts androgens to estrogens. Unlike tamoxifen, AIs are active in their parent form and deactivated by metabolism. Anastrazole, letrozole, and exemestane are few examples of AIs, that are found to block the activity of aromatase enzyme by 96-99%, leading to much lower endogenous estrogen levels than those seen in natural menopause in postmenopausal women (43).

The enzyme aromatase, responsible for synthesizing estrogen, is encoded by gene CYP19A1. Therefore, this gene serves as a target of interest and has considerable pharmacogenomic importance in inhibiting aromatase. SNPs in CYP19A1 have shown improved efficacy of AIs in the neoadjuvant and adjuvant settings (43). A study on AI-treated postmenopausal women in an adjuvant setting showed that two SNPs in the aromatase gene CYP19 caused significant change in aromatase activity after AI therapy.

Targeted therapy

Genetic variations in tumor cells influence drug metabolism, and have been recognized to select suitable therapeutic regimens to reduce resistance. With the advancement of pharmacogenomic approaches in breast oncology, targeted therapies have led to the implementation of novel drug regimens that confer maximum drug efficacy with minimum toxicity (51). Targeted therapies are generally implemented to treat patients who express several distinctive proteins on tumor cell surface, called tumor-associated antigens (TAAs), which promote abnormal growth patterns. For this purpose,

antibodies are mostly used as they function like the human immune system (52).

TAAs have been successfully targeted by novel drugs and bispecific antibodies. Nowadays, the most effective targeted therapy for BC focuses on inhibiting the overexpression of HER2 protein, found on the surface of BC cells. Trastuzumab is the first humanized monoclonal antibody to receive FDA approval against HER2 receptor in breast cancer (51).

Currently, there are seven widely used targeted therapies that effectively block various molecular pathways in BC (52):

1. Afinitor or everolimus: it is an m-TOR inhibitor that obstructs energy supplies of BC cells;
2. Avastin or bevacizumab: it diminishes the formation of new blood vessels and thus blocks the oxygen and nutrient supply of cancer cells;
3. Herceptin or trastuzumab: a monoclonal antibody that binds to HER2 receptor, inhibiting cell proliferation and halting their growth;
4. Kadcyla or T-DM1: it is a combination of Herceptin and emtansine. It transports emtansine chemotherapy to cancer cells;
5. Perjita or pertuzumab: it blocks the growth signals of cancer cells;
6. Tykerb or lapatinib: it is HER2 inhibitor that inhibits cell growth;

In general, TNBC patients respond to a targeted treatment comprising PARP1 inhibitors, taxol derivatives, and anthracycline chemotherapy. The patients who are resistant to anthracycline and taxane drugs may be treated with microtubule-stabilizing agents ixabepilone and capecitabine (52).

Cytotoxic chemotherapy

Evolving from single alkylating agents to multiple chemotherapy regimens, cytotoxic chemotherapy has made substantial progress in treating both advanced and early-stage BC. BC patients' therapeutic index to cytotoxic drug is unique for every patient, and pharmacogenomics (PGx) may play an important role in explaining individual differences of chemotherapeutic outcomes.

Antimetabolites

Capecitabine is an orally administered, third-generation effective drug that is a pyrimidine analog 5-flourouracil (5-FU). It is one of the best treatments for triple-negative BC patients and metastatic BC, where capecitabine has proved to remarkably improve overall survival rate (44). Capecitabine potentially halts tumor growth by inhibiting DNA synthesis in cancerous cells. However, its catabolism-related defects may result in drug accumulation and toxicity. 5-FU prodrug capecitabine is catabolized by enzyme dihydropyrimidine dehydrogenase (DPD), which is coded by gene DPYD. Pharmacogenomics studies revealed that the key enzyme responsible to convert capecitabine into 5-FU may undergo genetic deactivation and lead to drug toxicity (43).

Gemcitabine is an antimetabolite and nucleoside analog that brings about tumor apoptosis by inhibiting DNA replication. It is commonly used in the treatment regimens for metastatic and advanced BC. Gemcitabine-related hematologic toxicity, tumor response and survival rate are variable due to the SNPs variations in genes encoding drug metabolizing enzymes in BC patients (43).

Anti-microtubules

Taxanes are microtubule antagonists, considered as powerful cytotoxic agents, that efficaciously improve overall survival in adjuvant and neo-adjuvant chemotherapies in BC. They include paclitaxel and its semi-synthetic analog docatexel, which target microtubules and inhibit the dynamics of these mitotic spindles. This way, they cause cytotoxicity in tumors by blocking mitosis and triggering cell death in tumor cells (44).

Taxanes are hydroxylated in the hepatic CYP3A4 system. Paclitaxel is further metabolized by CYP2C8, and the genes encoding its metabolism undergo genotypic variations that influence the clearance rate of paclitaxel. Research about paclitaxel-containing regimes in BC patients showed variable treatment response: patients harboring the variant allele of this gene CYP2C8*3 are at increased risk of getting severe peripheral neurotoxicity. This taxane-induced peripheral neuropathy (TIPN) in BC patients can be devastating and treatment-limiting (43). Genes that are linked to cause TIPN serve as pharmacogenomic biomarkers that may alter treatment outcomes.

Anthracyclines

Anthracyclines make up a class of drugs that suppress enzyme topoisomerase II, thus causing apoptosis of tumor cells. Doxorubicin, epirubicin, daunorubicin, and idarubicin are different anthracyclines that are potent anti-tumor chemotherapeutic agents, extensively used in adjuvant and neoadjuvant settings, and showed improved survival rates. Their combination with cyclophosphamide (AC) is the foundation of chemotherapy regimens used to treat BC, which has replaced many other regimens due to its exceptionally favorable outcomes with BC patients (44).

A noteworthy limiting factor in consuming these potential cytotoxic agents is the so-called anthracycline-induced cytotoxicity (AIC), an adverse event that manifests as serious cardiotoxicity, hematological toxicity, gastrointestinal toxicity, and febrile neutropenia. Pharmacogenetic variations in enzymes that code for anthracycline metabolism and transport, and cause oxidative stress have been defined in the literature, but lack substantial evidence for association with AIC in BC. For this purpose, variants of carbonyl reductase (CBR1 and CBR3), a doxorubicin metabolizing enzyme, have been widely studied, but no significant association of genetic variation with AIC has been observed (43).

Cyclophosphamides

Cyclophosphamide is considered as a mainstay in almost all BC chemotherapeutic treatments. It is a DNA alkylating agent, that is a prodrug that undergoes hepatic metabolism,

primarily governed by CYP3A4, CYP2B6, and CYP2C9 genes (43). Cyclophosphamide metabolites exhibit significant variations in different patients' plasma concentrations, predicting that its metabolism is influenced by genetic variations. The clearance of cyclophosphamide is also under genetic control, and genetic variations in its pathway may be suitable pharmacogenetic targets. An enzyme that causes detoxification of cyclophosphamide metabolites is ALDH1A1, which has also been linked with poor prognosis of the basal-like breast cancer subtype (43). Its chronic use may induce cytotoxicity, i.e., hematological toxicity, nausea, vomiting, and reversible alopecia (44).

With the rapid development of pharmacogenomics and bioinformatics, the management of studies on pharmacogenomic biomarkers of BC has become much faster than in the past. Due to the diversity of patient responses to anticancer medications and their narrow therapeutic index, the above-described pharmacogenomic disciplines have scope to provide tailored oncological treatments for breast cancer. While the initial attention was given to protein markers, such as ER and HER2 receptors, a significant number of the latest predictive biomarkers employed to direct treatment decisions are from genomic origin.

Immunotherapy in breast cancer

TNBC, characterized by the absence of estrogen receptor, progesterone receptor, and HER2 expression, poses a formidable challenge in breast cancer treatment due to limited targeted therapeutic options. Immunotherapy has emerged as a promising strategy to address this unmet clinical need (53). Indeed, advancements in immunobiology have paved the path for effectively boosting host immunity in the fight against breast cancer. By leveraging the host immune system, immunotherapy aims to activate and enhance the antitumor immune response (53, 54). Immune checkpoint inhibitors, such as anti-PD-1/PD-L1 agents, have shown potential in unleashing T cell-mediated immune responses against TNBC cells, and increased antitumoral immune response in preclinical studies (54). Additionally, adoptive T cell therapies and therapeutic cancer vaccines are being explored to augment the antitumor immune response. While certain TNBC cases have exhibited remarkable responses to immunotherapy, challenges such as inherent or acquired resistance, tumor heterogeneity, and identifying predictive biomarkers necessitate further investigation (53, 54). To this goal, combining immunotherapy and conventional treatments could be a new way forward to enhance treatment efficacy.

Metabolomics of breast cancer

Metabolomics is the measurement of the aggregate metabolic outcome of biological systems, which establishes a reflection of dynamic cellular functions due to pathophysiological stimuli and genetic variations (55, 56). A metabolome is a quantitative collection of small molecules, called metabolites, present in a biological sample (blood, urine, saliva, serum) at a specific time. It is the representative of all cellular processes, and encompasses a wide range of compounds such as lipids, amino acids, sugars, nucleotides, and various other metabolites involved in cellular processes (57).

Metabolomics, viewed as a consequential stage of proteomics, transcriptomics, and genomics, offers promise as a potentially non-invasive liquid biopsy approach. In the future, it could be employed for cancer diagnosis and characterization, for monitoring treatment response and toxicity, as well as for predicting outcomes from the initial stages of diagnosis (55).

Metabolic fingerprint of a metabolome

A possible explanation of the connection between metabolome and genome (with the first being dependent on the latter) is as follows: one's genetic information is contained in the genome as DNA sequences, which are transcribed into RNA (transcriptome), which in turn is translated into a protein (proteome) that undergoes metabolism, ultimately forming small molecules, called metabolites, in a metabolome. Therefore, any genetic alteration—such as mutation, over-expression, deletion or insertion—may cause significant changes in the metabolic profile. These genetic changes that alter the metabolic profile also step up cancer development (56).

The metabolomic constitution is also influenced by various other factors, both from internal and external sources, including age, gender, race, diet, physical activity, health state, and drug exposure (58). Consequently, the distinct patterns of individually expressed metabolites form a unique metabolic fingerprint, indicating the idiosyncratic biological configuration of that individual's metabolic process (55).

In a wide-ranging study on metabolomics, operating 928 cell lines from more than 20 diverse cancer types, researchers discovered 225 metabolites that are distinctive to cancer metabolism. Similarly, substantial evidence links obesity and physical inactivity to an elevated risk of developing breast cancer (58). As alterations in metabolomic profiles can be linked to various pathological conditions, the study of the metabolomic biomarkers will play a crucial role in advancing personalized medicine as well as enabling early detection and biological characterization of diseases, especially cancers (55, 56).

The analysis of a metabolome is performed by three main techniques. These are nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), and liquid chromatography mass spectrometry (LC-MS) (56, 57). Moreover, scanning electron microscopy (SEM), matrix-assisted laser desorption ionization (MALDI), and nanostructure-imaging mass spectrometry (NIMS) have also been employed for metabolome investigation (56).

Metabolomic profile in breast cancer

Tumor cells exhibit notable deviations in cellular processes and altered metabolites compared to normal cells. Notably, altered metabolic pathways in malignant cells are widely acknowledged. One considerable example is the use of aerobic glycolysis instead of the typical mitochondrial oxidative phosphorylation to produce adenosine

triphosphate (ATP), a well-known phenomenon referred to as the "Warburg effect". This adaptation of cancer cells is believed to provide an advantage to survive even in hypoxic conditions (57).

It is evident that cancer cells require ample supply of nutrients for sustenance and growth. They use this energy for proliferation, angiogenesis and epithelial-to-mesenchymal transition (EMT) (56). Distinct metabolic profiles in different subtypes of BC also aid in their classification: for example, comprehensive analysis of glutamine to glutamate ratio in tumor tissue has revealed links with estrogen receptor status, tumor grade, and overall survival (57). Similarly, metabolites from other energy-generating pathways are found in higher levels in triple-negative BC than in hormone-receptor positive BCs, and it suggests aggressiveness (56).

Irrefutably, metabolomic analysis led to the discovery of potential biomarkers for early diagnosis and tailored therapy that warrant further validation.

Mitochondrial dysfunction in BC

Mitochondria possess their own genome, called mitochondrial genome (mtDNA, or mitogenome), whose transcription and translation are controlled by various mechanisms. It has been evidenced that there is an efficient epigenetic system—containing methylated DNA/RNA bases, a network of noncoding RNAs, and posttranslational mechanisms of histone modification—that controls gene expression of mtDNA. An established cross-talk mechanism has been observed between nuclear and mitochondrial genomes, which is also responsible to control the mitochondrial activity (59).

mtDNA is very vulnerable to damage by nuclear DNA mutations, leading to the malfunctioning of the mitochondria-operated respiratory chain and energy production and consequently promoting the generation of additional reactive oxygen species (ROS), which sponsors oncogenicity. Reliable studies evidenced that aberrations in mitochondrial genome are also involved in breast cancer origination and progression (56). Unstable mitochondrial epigenetics (mito-epigenetics) and defective governance of oxidative phosphorylation processes potentially foster the growth of cancer cells. Therefore, these alterations and mutations in mtDNA can be a powerful target for anti-breast cancer therapies. Different mitochondria-centered treatments have been tested in breast cancer clinical trials. These include OXPHOS inhibitors, antibiotic bedaquiline, biguanides, vitamin E analogs, etc. (59).

Lipid metabolism in BC

Cancer cells essentially rely on ample lipid supply and metabolism for their growth and proliferation: besides providing energy, lipids form the structural foundation of cell membranes, serve as energy reservoirs, and also act as signaling molecules. Cancer cells use fatty acids, which are like building blocks made of lipids, in two possible ways: either for lipogenesis (i.e., to synthesize many lipid molecules for tumor growth), or to transport many fatty acids into the cells to generate energy, to be used by cancer cells by beta oxidation. In numerous cases, the presence of metabolites responsible to cause fatty acid metabolism and transport is indicative of breast cancer, and they can serve as biomarkers for early detection (56, 59).

Another lipid metabolic alteration is the presence of higher acylcarnitine C2 levels, which is associated with increased risk of breast cancer. Acylcarnitine C2 facilitates the transfer of fatty acids into the mitochondria and its abundance is an indicator of excessive lipid availability and heightened fatty acid oxidation in breast cancer (58).

There is an association between cancer cells and adipocytes that favors oncogenesis. Adipocytes secrete a hormone, called leptin, which—like insulin—was found to facilitate breast cancer growth, thus making hyperleptinemia an important metabolic indicator in the pathophysiology of breast cancer (61). Similarly, high levels of CD36, a fatty acid transporter that facilitates the influx of exogenous fatty acids, exhibit increased protein expression in various cancer types, including BC (60). In light of these findings, it is apparent that BC cells show eminent dependency on fatty acid and lipid metabolism to grow and multiply. Hence, the aforementioned metabolic pathways can be pharmacologically inhibited to mitigate breast cancer.

Carbohydrate metabolism in BC

Cancer cells use carbohydrates as their primary energy source, but they prefer aerobic glycolysis for glucose metabolism, even when the oxygen exists (Warburg effect). Increased glucose uptake by cancer cells is due to the overexpression of oncogenes RAS and MYC, and mutation in tumor suppressor gene TP53, which leads to high proliferation and decline of apoptosis (62). Elevation in various processes like glycolysis, glycogenolysis, redox pathways, and TCA cycle is not just for increased energy production, but they also release certain metabolites that act as precursors for many macromolecules (56): for example, glucose-6-phosphate metabolizes to give precursor molecule ribose-5 -phosphate for nucleic acid biosynthesis, and intermediate 3-phosphoglycerate is a precursor for amino acids glycine and cysteine. So, excess generation of precursor molecules promotes cancer biomass and proliferation (56).

Glucose transporter proteins (GLUT1-5) are highly expressed in breast cancer; specifically, GLUT-1 is excessively expressed in TNBC patients (63). Nowadays, several GLUT-1 inhibitors (like BAY-876) have been implemented as potential targeted therapeutics to specifically inhibit TNBC cell lines (64). Additionally, excessive release of lactate in the tumor microenvironment, as a result of Warburg effect, makes the microenvironment acidic, which eventually encourages tumor progression, angiogenesis, metastasis, and essentially immunosuppression, thus leading to adverse outcomes (65, 66). Clinical investigators found out lactate to be an oncometabolite, as it amplifies the expression of genes involved in cell division, cell proliferation, and elevated transcription in human breast cancers (66).

Briefly, carbohydrate metabolites play a major role in amplifying breast cancer and demand researchers' attention to develop targeted therapies (56).

Amino acid metabolism in BC

Like any other cancer, breast cancer also uses amino acids for cell proliferation and persistence. Collectively,

essential, semi-essential, and non-essential amino acids play key functional roles inside the cells, such as epigenetic modifications, α-ketoglutarate production (which acts as a substrate for TCA cycle), ATP production, protein synthesis, glucose and lipid metabolism, and signaling pathways (56).

In this regard, glutamine and its intermediate metabolites (NADH and glutathione) are vital for cancer growth, as they meet energy demands and help in combating oxidative stress in tumors. Sometimes cancer cells undergo glutamine addiction, because they are unable to survive when glutamine is lacking (63). Therefore, cancer cells exhibit increased expression of glutamine transporters ASCT2, SNAT1, SNAT2, and SNAT5 for its sufficient influx. This crowded glutamine enters the cell, first gets converted into glutamate, and then undergoes metabolism through TCA cycle to generate an immense amount of energy for cancer cells by the process of glutaminolysis (56). This process also releases some macromolecules that cancer cells use when lacking glucose—like citrate, malate, and fumarate. Reduced glutamine metabolism encourages lipid biosynthesis, which in turn favors tumor cells in hypoxia or mitochondrial dysfunction. Therefore, glutamine promotes carcinogenesis, even when nutrients are inadequate in the microenvironment. The key enzyme glutaminase, converting glutamine into glutamate, is a potential target for breast cancer treatment (67). Its inhibitors have given successful results in diminishing tumor growth in TNBC cell lines. In addition, the inhibition of ASCT2 (a glutamine transporter) also turned out to be successful in halting tumor growth in TNBC patients (56).

Similarly, serine transporter ASCT1 is also highly expressed in breast cancers. Cancer cells highly depend on the availability of extracellular serine, which they use for nucleotide synthesis and DNA methylation. Moreover, an overexpression of serine biosynthesis genes is also associated with breast cancer metastasis to bones and stimulates osteoclastogenesis (68). Minimization of serine levels can prevent the growth of cancer cells.

Likewise, homocysteine, cysteine, and branched chain amino acids like leucine, iso-leucine, and valine are also crucial for cancer cell proliferation. Especially, a cysteine excess can cause oxidative damage and produce free radicals, which become sources of gene mutations. Additionally, higher plasma cysteine levels indicate the risk of breast cancer and helps in early diagnosis (69). Tryptophan and L-arginine disturbs immune regulation and potentially promotes the growth of breast cancer cells, and thus their suppressants contribute to halt the tumor growth (63). Targeting amino acids metabolism could thus be useful in preventing and treating breast cancers.

One of the hallmarks of cancers is metabolic reprogramming, which allows researchers to predict whether the initially observed premalignant lesions may proliferate or metastasize in future. This creates opportunities to pinpoint the metabolic dependencies of BC, in order to overcome its pathogenesis and overall burden.

Differential metabolomic fingerprints for breast cancer subtypes

Alterations in the gene expression profile cause changes in the metabolic profiles of BC subtypes. For example, luminal subtypes emerge by alterations in GATA3 and PIK3CA mutations, whereas TP53 mutations give rise to basal-like BC (12). Therefore, each BC subtype exhibits variations

Table 2. Metabolic signatures in various molecular subclasses of breast cancer

	Luminal A	Luminal B	HER-2	Basal-like (TNBC)	Reference
IHC status	(ER+) (PR+) (HER2-)	(ER+)(PR+)(HER2+)	(ER-)(PR-)(HER2+)	(ER-)(PR-)(HER2-)	
Glucose metabolism	High glucose consumption, high lactose production, poor TCA activity, slow glycolysis, locally multiplied cells	Low glucose consumption, high lactate production, efficient glycolysis, aggressive cancer	Increased glucose consumption, high lactate production, enhanced glycolysis, low oxygen consumption, more aggressive than luminal subtypes	Higher glycolysis, high lactate accumulation, lower oxidative phosphorylation than luminal subtypes, highly invasive breast cancers	(70)
Amino acid metabolism	Low expression of GDS and GLD, decreased glutamate-to-glutamine ratio	High expression of GDS and GLD, decreased glutamate-to-glutamine ratio	High expression of GDS and GLD, higher glutamate-to-glutamine ratio, active glutaminolysis	Higher glutamate-to-glutamine ratio, active glutaminolysis	(71)
Lipid metabolism	Upregulation of de novo FA synthesis, mobilization, and oxidation	Upregulation of de novo FA synthesis, mobilization, and oxidation	Highest expression of lipid metabolic proteins, increased de novo FA synthesis	Expression of lipid metabolism protiens is slightly lower than other subtypes, increased de novo FA synthesis	(72, 73)

Abbreviations: ER, estrogen receptor; PR progesterone receptor; HER2, human epidermal receptor2; TNBC, triple-negative breast cancer; TCA, tricarboxylic acid; GDS, glutamate dehydrogenase; GLD, glutamate decarboxylase; FA, fatty acid.

in metabolic alterations or metabolic signatures that can be helpful in predicting the possible therapeutic strategy. Moreover, metabolomic analysis and subtype differentiation can be simply performed through plasma samples, which sidesteps the hustle of performing a biopsy.

In this context, the distinct metabolic fingerprints of each subtype are now presented in **Table 2**. This compilation is made by extensive review of studies and could serve as a comprehensive reflection of the metabolic profile of each subtype.

Microbiomics of breast cancer

Human bacterial composition causes noticeable alterations in the normal functions of the body, such as metabolic changes, inflammation, allergy, and cancer progression. In the recent years, much attention has been given to the characterization of the microbiota from different parts of the body—including gut, skin, urinary tract, and other organs—to correlate their potential in stimulating carcinogenesis. Each organ exhibits a distinct microbiota, which leads to well-defined pathological findings, including cancer (74). Any alteration in the microbiome ecology of each specific organ leads to **"dysbiosis"**, a phenomenon that causes disease, pathological conditions, and tumorigenesis (75).

Currently, research on breast microbiota has discovered a unique bacterial population, which is important for breast health because these bacteria may possess either pro- or anti-carcinogenic properties. Next generation sequencing (NGS) techniques are a powerful tool in revealing bacterial signatures in breast health and their role in carcinogenesis. Additionally, they may serve as important biomarkers and targets for BC therapies (76).

Mechanisms by which dysbiosis induces breast cancer

Several pathways have been discussed in the literature, through which breast tissue microbiomes can induce tumorigenesis.

The first possible way is the incitement of chronic, dysregulated inflammation, which can lead to malignancy. Dysbiosis destroys the host's immune regulation and leads to tumor-promoting inflammation. Studies have shown that cancer patients show a decreased lymphocyte count, associated with disease relapse and high mortality (73, 75).

Secondly, the diverse microbial population in human gastrointestinal (GI) tracts is responsible for influencing estrogen metabolism. Endogenous and circulating estrogen is the key hormone accredited to cause breast cancer. After performing its assigned roles in sexual cycle regulation, estrogen is inactivated by the liver and excreted through the intestines. However, certain bacteria present in the gut are able to deconjugate the hormone by the activity of enzymes β-glucoronidase and β-glucosidase, and free estrogen is absorbed again into the blood. Its abundance in circulation is linked with increased risk of breast cancer, especially in postmenopausal women (74, 75, 77). Researchers coined the term 'estrobolome' for all enteric bacterial genes that influence oestrogen metabolism. This estrobolome can be helpful in prognosis of ER-positive BC, and thus can be targeted via appropriate antibiotics (75, 77).

Moreover, an unhealthy diet with high fat and low fiber, along with dysbiosis in the gut microbiota, also leads to obesity, which is a potential risk factor for BC. Studies have shown that certain bacteria, like *Firmicutes* and *Bacteroidetes*, bring about metabolism of dietary fibers and polyphenols. Their decline in the GI-tract could lead to obesity and increased estrogen levels (75).

Next, some microbes are able to cause genomic instability and double-stranded breaks in DNA by producing colibactin, a genotoxin produced by certain *E. Coli* strains. This indicates complex interactions between gut bacteria and breast cancer (78).

Few studies also examined the difference between the microbiomes of BC patients and healthy controls. One study revealed that non-cancerous women harbor *Methylobacterium* in larger amounts in breast tissues than their BC counterparts. Moreover, urine sample comparison between the groups revealed differences in microbiomic profiles: cancer patients were found to carry more abundant gram-positive bacteria in their urinary tract than the control group (79, 80).

However, even though numerous studies demonstrate correlations of bacteria with BC, their exact causative roles are still unclear, and the precise mechanisms powered by such bacteria are still to be fully understood.

Microbiome and bacterial therapy for breast cancer

Classical treatments to exterminate breast cancer include surgery, radiotherapy, chemotherapy, and some modern approaches like immunotherapy, stem cell therapy, dendritic cell-based therapy, hormonal therapy, and so on. However, all of these methods have their own limitations in clinical practice. For instance, chemotherapy induces nonspecific toxicity towards the host's normal cells, and also their multidrug resistance. This is why genetic engineering has yielded genetically modified non-pathogenic bacterial strains that are selective for cancer cells, with lower toxicity and fewer side effects. Bacteria-mediated tumor therapy (BMTT) is a potential therapeutic approach for breast cancer, on the way of its refinement, which is gaining the attention of researchers and giving promising results (81, 82).

Previously, the role of carcinogenic bacteria has been extensively reported in the literature, highlighting their capability to enhance tumor progression. However, recently, some bacterial species have demonstrated great potential to invade and colonize solid tumors, resulting in growth retardation and, occasionally, even complete eradication of cancer. Examples of such strains include *Clostridia*, *Bifidobacteria*, *Shigella*, *Vibrio*, *Escherichia*, and *Salmonella* (81, 82).

Bacteria can hinder with tumor progression in many ways, some of which are listed below:

Bacteria like *E. Coli* are used for host immune response stimulation. Stimulated T-lymphocytes are associated with antitumor activity (81);

Bacterial products like toxins, bacteriocins, and enzymes produced by specific bacterial strains have shown oncolytic properties. For example, bacteriocin Bovicin HC5 demonstrated anti-cancer activity in breast cancer cell lines in vitro.

Additionally, Laterosporulin 10 (LS10) is a peptide produced by gram-positive bacteria that can induce necrosis and cell death in breast cancer cell line MCF-7 (81);

Sometimes attenuated strains of bacteria (e.g., *Salmonella*) have also been used for cancer bacteriotherapy, in which bacteria are used as vehicles to target human tumors. For example, attenuated bacteria of *Salmonella typhimurium* produce antitumor activity against different cell lines of breast cancer (81).

Conclusions

Interest in the multi-omics approach to study breast cancer has increased in recent years, as it has become discernable that the relationship of genome, proteome, metabolome, and microbiome in BC can reveal novel biomarkers and therapeutic targets. Moreover, omics studies led to the discovery of specific genomic and metabolomic features of BC that are helpful in categorizing BC patients and identifying disease progression and response to treatment. For instance, the unique metabolic profile of BC patients is highly sensitive to the microbiota of breast tissue microenvironment. Therefore, analysis of metabolic changes in BC with respect to micro-biomic implications can provide new insights into treatment modalities. In conclusion, multi-omics has emerged as an innovative, promising approach for profiling specific omics features associated with BC, and demands solicitous large-scale future research.

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Conflict of Interest

Authors declare no conflict of interest.

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Exploring the Impact of Tobacco Usage on Microbiome Dysbiosis and Associated Health Risks: A Comprehensive Review of Recent Advancements and Future Directions

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Abstract

All over the world, tobacco usage is quickly expanding. Though it presents a major health risk and is anticipated to have long-lasting impacts on the public and economic health of the country, its consumers are increasing with every passing day. Tobacco is being used in a variety of ways, with cigarettes being the most popular. Smoking affects the healthy oral, intestinal, and pulmonary microbiomes, often altering the dynamic equilibrium of the diverse bacteria that make up the human microbiome, or "dysbiosis". Smoking-induced dysbiosis can lead to developing conditions like asthma, chronic obstructive pulmonary disease, Crohn's disease, ulcerative colitis, and periodontitis. The purpose of the following article is to provide a better and more comprehensive overview of the key areas that the tobacco industry needs to investigate, such as microbiome manipulation, to provide a complete picture of recent advancements in tobacco research while also keeping public safety in mind, and the various diseases linked to tobacco use. ***Clin Ter 2023; 174 Suppl. 2 (6):119-125 doi: 10.7417/CT.2023.2478***

Key word: *Smoking, tobacco, sublingual tobacco, oral microbiome, dysbiosis, public safety, health concerns.*

Introduction

Tobacco use has grown and diversified substantially since its introduction in Europe, in the 15th century. Until the 18th century, the most widespread forms of tobacco intake were smokeless or sublingual tobacco (also known as snuff) and pipe smoking. Nowadays, approximately two billion individuals worldwide consume tobacco products, predominantly by smoking cigarettes—which emerged in the nineteenth century and has gained in popularity ever since (1). Moreover, it has been observed that annually a minimum of four million individuals across the globe suffer from diseases associated with tobacco use (2), which include chronic obstructive pulmonary disorder, (3, 4) cardiovascular disorders, (5, 6), autoimmune diseases (7, 8), and numerous forms of cancer, imposing a significant financial burden on the healthcare sector (9, 10).

Despite cigarettes being the most widely used tobacco product, there are still many alternative ways to consume tobacco: some of these have been around since the days before cigarettes were widely available and have managed to survive and keep a substantial user base ever since (11,12). On the other hand, also new alternatives have been developed in the last years: for instance, vaping (that is using electronic cigarettes or e-cigarettes) and heated tobacco products are two new ways to consume nicotine that have been created in response to the significant rise in smoking-related mortality (2, 13). These new forms of usage, which are promoted or portrayed as being safer or harmless, have attracted both the new generations and long-time smokers (14-16). Table 1 presents a list of commonly utilized tobacco forms.

The prevalence of tobacco usage among young individuals is widespread globally. Apart from adults, also children and young individuals in the US face a significant health hazard, which is expected to have enduring consequences on the social and financial well-being of the nation (17). It is indisputable and well-documented that tobacco accounts for 75% of healthcare expenditures in the US (18). Primarily, the initiation of tobacco consumption typically occurs

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Table 1. Various ways to consume tobacco for daily use

Product	Definition	pH
Cigarette	Paper-rolled tobacco for smoking.	5.5-6 (acidic)
Cigar	The process of air-curing and fermenting tobacco is followed by the use of a wrapping material that incorporates tobacco leaf in various manifestations.	Products might have a buccal or inhalable pH between 6.5 (acidic) and 8.0 pH (alkaline)
Blunt	Cigarillo shells held cannabis.	-
Heated tobacco	Electronic devices generate heat in order to produce an aerosol by treating reconstituted tobacco sticks with a humectant (glycerin).	5.5-6 (acidic)
Chewing tobacco	The act of placing tobacco between the lip and gum or inhaling it through the nasal passage.	Range from more acidic (5.2-7.1) to more alkaline (pH 7.6-8.6)
Waterpipe/hookah	The process involves using charcoal to heat flavored tobacco, which subsequently undergoes a cooling effect upon traversing a chamber filled with water.	3.8-5.8 (acidic)
Electronic cigarettes	Devices that use electricity to turn a liquid into a spray, usually containing flavorings, propylene glycol, and nicotine.	7-9 (alkaline)

during teenage years and adolescence (19): over 88% of adult daily smokers affirm that they initiated their smoking habit before reaching 18 (20). During this developmental phase, individuals exhibit heightened vulnerability to social influences, including the persuasive tactics employed by tobacco advertisements, which exert a disproportionately adverse effect on the vulnerable minds of the youth (21). According to Fagerström and Doll (YEAR), cigarettes are the exclusive consumer product globally recognized for its association with premature mortality, affecting around 50% of those who smoke regularly (22, 23). Furthermore, this epidemic continues to affect the US, as well as other countries with lower or moderate incomes, which are not equipped to handle the resulting health and economic consequences (24, 25).

Besides addressing Public Health issues, the tobacco industry works constantly for innovating and looking into ways to make tobacco use safer and develop substitute behaviors. New research directions must be investigated to accomplish these goals. Therefore, the following article aims to present a better and more thorough overview of the important areas that the tobacco business needs to explore—including microbiome manipulation—to give a thorough picture of recent developments in tobacco research while keeping public safety in view, and the different diseases associated with the usage of tobacco.

Tobacco Use and Microbiome Dysbiosis

Cigarette smoking or any other form of tobacco use have been associated with several severe illnesses (26); additionally, they can cause the colonization of pathogenic bacteria by altering immune responses and the microbial communities that are connected to human beings, which are sensitive to many environmental factors (27-29). Smoking can affect the human microbiome, which includes different groups of microbes like viruses, protozoa, bacteria, and fungi, in different diseases (30). The human microbiome has the ability to maintain homeostasis in any case of disturbance, which can be influenced by factors like alcohol, antibiotics, smoking, and diet (31). Table 2 displays the variations in the microbiome across various species.

Many clinical disorders and health conditions have been associated with various constituents of tobacco, including fine particulates, chemicals, and heavy metals (41-48). Recent studies have reported that the diseases linked to smoking can be caused by the microbes present in tobacco. The microbes that have been identified in tobacco flakes, fresh tobacco leaves, or fine tobacco particles are *Acinetobacter calcoaceticus*, *Pantoea agglomerans*, and species of Pseudomonadaceae like *Stenotrophomonas maltophilia* and *P. fluorescens*; these microbes have been studied because of the DNA sequencing technology, which help in their identification through microbes' culturing (47).

Tobacco has the ability to suppress our immune system: it can lead to impairment of the bacterial community in the smoker's organism and thus affect their antimicrobial defenses. Tobacco smoking can impact the peripheral immune system by decreasing the action of natural killer cells and increasing the number of leukocytes in the body, thus increasing its vulnerability toward infection (49). Research has shown that smoking impacts the functionality of neutrophils and macrophages, leading to a reduction in dendritic cells and increased numbers of lymphocytes, macrophages, neutrophils, and eosinophils. Dendritic cells are integral components of the immune system, fulfilling crucial functions (50, 51).

The Role of Tobacco in the Development of Periodontal Disease

A considerable body of research indicates a strong association between the occurrence of periodontal disorders and both tobacco use and the highly complex microbial populations residing inside the subgingival sulcus (52-54). Tobacco smokers had a notably elevated susceptibility to the development of severe periodontal disease (55). Current research examined the microbial configuration of patients

Table 2. Alterations in the diversity of the smokers' microbial community

	Source	Specimen	Enriched microbes	Diminished microbes	References
Oral	Human	Subgingival plaque	Species: Pseudoramibacter alactolyticus, Fusobacterium nucleatum, Pseudomonas pseudoalcaligenes, F. naviforme, A. haemolyticus, Filifactor alocis, A. baumannii, Dialister microaerophilus, A. schindleri, Desulfobulbus sp. Clone R004, A. guillouiae, Megasphaera sueciensis, Acinetobacter johnsonii, M. geminatus, M. micronuciformis, M. elsdenii	Species: Hemophilus parainfluenzae, Streptococcus sanguinis, Neisseria subflava, S. parasanguinis, A. dentalis, S. oralis, A. israelii, Granulicatella elegans, Actinomyces viscosus, G. adiacens	(32)
	Human	Oral wash samples	Genera: Streptococcus, Atopobium, Lactobacillus, Bifidobacterium		(33)
	Human	Mouth wash sample	Genera: Atopobium, Treponema, Prevotella, TG5 and Mycoplasma, Porphyromonas, Megasphaera, Paludibacter, Dialister Phyla: Bacteroidetes and Actinobacteria, Spirochaetes, Synergistetes and Tenericutes	Genera: Leptotrichia, Neisseria, Fusobacterium, Eikenella, Lautropia, Aggregatibacter, Haemophilus, Actinobacillus Phyla: Cyanobacteria, Proteobacteria, GN02, Fusobacteria and SR1	(34)
Airways	Human	Oropharyngeal and nasopharyngeal swabs	Nasopharynx Genera: Eubacterium spp. Eggerthella, Anaerovorax, Dorea, Erysipelotrichaceae I.S. Oropharynx Genera: Veillonella spp., Megasphaera	Nasopharynx Genera: Shigella spp. Oropharynx Genera: Neisseria spp., Capnocytophaga, Fusobacterium	(35)
	Mice	Lung	Genera: Oxalobacteraceae, Escherichia-Shigella, Trichococcus	Genera: Caulobacteraceae-unclassified, Oceanospirillales, Raoultella, Lactobacillus, Caulobacteraceae-Phyllobacteriaceae-uncultured, Lactobacillaceae, Enterobacter, Acidimicrobiales-norank	(36)
	Human	BALF	Virome: Haemophilus, Rhodoferax phages, Prevotella, Capnocutophaga, Xanthomonas, Aeromonas and Actinomyces	Virome: Spiroplasma phages, Lactobacillus, Enhydrobacter, Gardnerella phages, Morganella, Enhydrobacter, Holospora and Enterobacter	(37)
Gut	Rat	Caecal contents	-	Genera: Bifidobacterium sp.	(38)
	Mice	Caecal contents	Genera: Clostridium sp.	Genera: Segmented filamentous bacteria, Lactococcus sp., Enterobacteriaceae sp. and Ruminococcus sp.	(39)
	Mice	Colonic sample	Genera: Lachnospiraceae sp.	-	(40)

who suffer from moderately to severe prolonged periodontitis in smokers. The results of the study revealed significant variations in the prevalence of disease-causing bacteria and those that are linked to oral health. Specifically, there was a higher prevalence of *Fusobacterium*, *Treponema*, *Bacteroides*, *Parvimonas*, and *Campylobacter*, while *Veillonella*, *Streptococcus*, and *Neisseria* were found to be at lower levels (56). Many different mechanisms contribute to the increased risk, progression, and severity of periodontal conditions in smokers.

There are several elements that contribute to the negative impacts of tobacco use on dental health. Initially, tobacco use leads to decreased blood flow to the gums, resulting in limited delivery of essential nutrients and oxygen and impaired removal of waste products. Secondly, tobacco use suppresses the immune response, particularly the inflammatory response, which is crucial in maintaining oral health. It also hinders the periodontium's ability to recover, both structurally and functionally. Lastly, disrupting the balance of oral microbiota subsequently leads to heightened vulnerability to diseases. The confluence of these several factors hinders the process of wound healing, thus making the progression of periodontal disease faster (57).

Probiotic Interventions and Harm Reduction Perspectives

Within the broad spectrum of reported results, in vitro studies have demonstrated that: (i) The regulation of the immune system and inflammation is influenced by exposure to periodontal pathogens, which in turn affects the development of cellular mediators; (ii) There is an hindrance in the growth

and attachment of infectious organisms to dental surfaces; (iii) The release of diverse antimicrobial agents modifies the surrounding environment (58, 59).

A potential player in overcoming microbiome dysbiosis could be a probiotic called *Lactobacillus reuteri* (*L. reuteri*), which has the ability to secrete certain antimicrobial compounds, including lactic acid, reuterin, and nitric oxide (NO) (60, 61). In particular, the last compound has bactericidal properties against anaerobic strains *F. nucleatum* and *P. gingivalis* (62, 63). Moreover, the antimicrobial molecules show a broader spectrum of inhibitory activity, including many types of microorganisms, like fungi and protozoa (64). Lactic acid bacteria can produce bacteriocins, bioactive chemicals that demonstrate inhibitory properties against particular periodontopathogens. There are examples in literature that show their use in the treatment of periodontitis, regardless of the concurrent use of chlorhexidine, which is a wide-spectrum antimicrobial substances (65, 66). Probiotics induce alterations in the activity of immune cells, thus indirectly influencing their impact on periodontopathogens: one such effect is the stimulation of macrophages to produce reactive oxygen species (ROS) (67). The presence of certain factors may result in adverse effects on anaerobic microorganisms, like *P. gingivalis*, that thrive in low oxygen conditions (68). Additionally, *L. reuteri*'s anti-inflammatory and cytokine-secretion-inhibiting properties are likely to be responsible for its positive impact on periodontal infections (69-71). The potential anti-inflammatory effects of *L. reuteri* may contribute to the regulation of the matrix metalloprotein and tissue inhibitor of metalloprotease-1 balance, as well as the inhibition of pro-inflammatory cytokines. These effects may potentially mitigate inflammatory processes and the degradation of periodontal tissues (72).

Harm reduction measures in the context of tobacco smoking is a subject that elicits different points of view. There are apprehensions over the potential impact of promoting smoking reduction on individuals' long-term cessation motivation. It is suggested that such efforts may inadvertently create a setting that would be beneficial to the promotion of "reduced risk" products by the tobacco industry, as well as to conducting biased studies aimed at demostrating their efficacy (73). The primary focus in addressing the negative consequences linked to tobacco smoking should be on promoting smoking cessation as the most impactful strategy (74). Using tobacco-related products in a way that is less detrimental than conventional goods (potential strategies to mitigate the harmful effects of cigarettes include employing novel curing methods to decrease tobacco-specific nitrosamines, incorporating catalytic agents to minimize the production of aromatic polycyclic hydrocarbon cancerous substances in smoke, using genetically altered crops to minimize nicotine or nitrosamine content, or implementing filtering techniques in order to selectively decrease the overall quantity of toxic substances) and pharmacological interventions (tools that resembles cigarettes, such as ones that heat up instead of burning their tobacco content) to diminish tobacco consumption or mitigate associated health risks (75).

Several of the chemicals used to develop drugs in pharmaceutical companies have been linked to negative consequences and significant expenses (76, 77). Therefore, natural products like curcumin, propolis, aloe vera, and honey have garnered significant attention from the nutritional and pharmacological sectors. This is due to their easy accessibility and potential use in cost-effective therapies with minimum or no toxicity, in contrast to traditional techniques (78, 79). The abundance of bioactive chemicals has several benefits, such as anti-inflammatory, anticancer, antimicrobial, anesthetic, and wound-healing effects. These compounds can potentially disrupt multiple cellular signaling pathways, which could influence the development of oral mucositis and the behavior of cancerous cells (80).

Polyphenols have emerged as promising natural therapeutic agents for combating oral infections (81). Olives contain significant quantities of phenolic chemicals, with varying concentrations that range from 1% to 3% of the olive's total weight in its fresh state. Olives include a diverse array of phenolic chemicals, including flavonoids, phenolic alcohols, secoiridoids, and phenolic acid, with the latter being of particular significance. Phenolic compounds like tyrosol and hydroxytyrosol have the most significant levels of occurrence inside olives, and are very interesting for their antioxidant, anti-inflammatory, and anti-microbial activities (82). According to previous reported studies, olive leaf extract intake resulted in a significant reduction in oral mucositis, which may be attributed to the observed decrease in levels of IL-1β and TNF-α in saliva (83). Olive leaves contain polyphenols that exhibit anti-inflammatory properties and provide protection to DNA against oxidative damage caused by free radicals. Regular use of extra virgin olive oil, which contains many omega-3 fatty acids and monounsaturated fatty acids, has been shown to decrease the occurrence of macrovascular problems and to suppress the synthesis of inflammatory proteins, including interleukin-6 and C reactive protein. There is a need for more investigation into the molecular processes that are potentially responsible for the protective effects of polyphenols in relation to numerous health issues (84).

Conclusions

Maintaining high levels of research and innovation within the tobacco industry is essential for enhancing the health benefits of non-smoking consumption methods and for reducing smoking-associated risks. Microbiota manipulation opens up promising new possibilities for reducing the harmful effects of tobacco-related substances and for improving smokers' health.

Future Prospects

The increasing emphasis on fitness and balance compels us to reassess our stance towards cigarettes and other excesses. It is impossible to overemphasize the importance of tobacco-related research and development. We can try to create less harmful options for smokers by learning from other sectors' successes, like the availability of Coca-Cola Zero. The use of novel technologies and natural substances can reduce tobacco product toxicity and prioritize consumer health. The introduction of biodegradable cigarette filters, for example, is an excellent illustration of how research

may play a significant role in mitigating the adverse consequences of tobacco use, while also ensuring environmental sustainability. Using such filters primarily aims at mitigating the adverse environmental impacts associated with removing cigarette butts by utilizing eco-friendly materials, specifically plant-based fibers. Preliminary investigations have yielded encouraging findings, demonstrating notable decreases in carcinogenic substances and other detrimental components inside filtered smoke. Additional investigation and improvement of these filters will have the potential to enhance the safety of smoking for individuals, and will be able to address environmental issues linked to tobacco waste. The potential advantages of natural substances in enhancing oral well-being have also received considerable study. According to existing research, integrating these natural components into oral sprays or alternative delivery systems can offer a safeguarding effect against oral health problems caused by smoking. Additional research will help in better understanding the appropriate dosage, effectiveness, and impact of these substances.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Original article

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Reduction of nitrosamines in cigarette smoke vapors through a filter functionalized with polyphenols from olive tree

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Abstract

Objective. In our study, we present the development of a novel cigarette filter enriched with polyphenols, with a particular focus on hydroxytyrosol extracted from olive sources. Our objective was to trap the presence of carcinogens in cigarette smoke by chemically modifying the filter surface.

Materials and methods. To evaluate the filtration efficiency of the newly developed filter, we employed an automated Stain Pattern technique, enabling non-intrusive measurement of behavioral vent blocking. The surface modification of cigarette filters was meticulously carried out to target the reduction of nitrosamines formed during combustion.

Results. Our extensive investigation underscores the potential of functionalizing cigarette filters using olive polyphenols, in particular hydroxytyrosol to mitigate the formation of harmful compounds, particularly nitrosamines, during smoking. Functionalized filters exhibited remarkable filtering efficiency, as evidenced by a capture factor (f=2.9×103) for two layers.

Conclusions. This innovative approach has the capacity to revolutionize the utilization of filters in commercial cigarettes, significantly reducing consumers' exposure to toxic chemicals. Our research demonstrates that hydroxytyrosol-functionalized cigarette filters can effectively remove noxious substances like nitrosamines, offering a promising avenue for enhancing public health.

Further in-depth research is essential to assess the protective impact of hydroxytyrosol-functionalized filters cigarettes, ensuring their potential to safeguard consumers' health effectively. ***Clin Ter 2023; 174 Suppl. 2 (6):126-141 doi: 10.7417/CT.2023.2479***

Key words: *Maillard Reaction, Advanced Glycation End-products, Nitrosamines, Cigarette Smoke, Filter Functionalized, Methylglyoxal, Polyphenols, Olive Tree, Hydroxytyrosol.*

Introduction

The Maillard reaction is a widely recognized chemical process in the realm of culinary arts. It brings about alterations in the color and sensory qualities of food, as well as changes in functional attributes and digestibility of proteins. Maillard reactions start with the joining of amino groups—present in proteins, peptides, and amino acids—with carbonyl groups from reducing sugars. This union leads to the formation of Schiff bases, which are then rearranged to produce Amadori or Heyns products (1,2).

This intricate reaction generates a range of molecules, including advanced glycation end-products (AGEs). AGEs are modifications occurring on Lysine (Lys) residues and cross-linked compounds originating from both Lysine (Lys) and Arginine (Arg) residues.

Several of these molecules—such as N-e-(carboxymethyl) lysine (CML), N-e-(carboxyethyl)lysine (CEL), pyrrolidine, methylglyoxal-lysine dimer (MOLD), glyoxal-lysine dimer (GOLD), and pentosidine—have been associated with potentially undesirable side effects. AGEs have been implicated in inflammatory conditions and may contribute to the progression of various diseases, including renal failure, diabetes, chronic heart failure, atherosclerosis, and Alzheimer's disease.

Methylglyoxal (MGO), a reactive carbonyl compound generated as a result of the Maillard reaction, has been investigated for its potential role in AGE formation as well as its antimicrobial properties (3). Consequently, it is established that the Maillard reaction gives rise to these AGEs, specifically α-dicarbonyl compounds, which have significant implications for health and various medical conditions.

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Glyoxal (GO) and methylglyoxal (MGO), which originate from Amadori Products and are integral components of AGEs as illustrated in Figure 1, represent some of the most harmful compounds released in both electronic cigarette (E-cig) and conventional tobacco cigarette smoke. These toxic compounds lead to excessive mucus production, a major pathophysiological feature of airway diseases (4).

However, as indicated in the literature (3,5,6), it is worth noting that MGO molecules can be effectively captured or shielded by polyphenols. The use of functional ingredients—like plant polyphenols, vitamins, and enzymes—has been explored as a strategy to regulate Maillard reactions in food (3). In 2005, research by Totlani and Peterson (6) demonstrated that Epicatechin, a polyphenolic compound found in plants such as green tea, grapes, and cocoa, can effectively capture α-dicarbonyls. The use of polyphenols from various plant sources as inhibitors of Maillard reactions has gained increasing attention in the realm of food systems. This is primarily due to the fact that these compounds are natural in origin and are therefore more readily accepted as food ingredients compared to synthetically manufactured alternatives.

In the culinary tradition of the Mediterranean diet, olive oil, renowned for its richness in polyphenols, is among if not the most frequently used condiment (7). Building upon this premise, the study conducted by Troise et al. delves into the impact of olive mill wastewater polyphenol powders (OMWPs) on the formation of dietary advanced glycation end-products (d-AGEs), dicarbonyls, and acrylamide in cookies (5). Its findings reveal that OMWPs, particularly when derived from secoiridoids-based functional ingredients, exhibit a notable ability to mitigate the formation of d-AGEs, acrylamide, and other products derived from the Maillard reaction in both model systems and cookies (5). It is thus evident that the polyphenols present in olive oil possess the capacity to intercept specific molecules generated by the Maillard reaction, some of which are also present in cigarette smoke.

Consequently, our research aims to investigate the potential of the use of Hydroxytyrosol (HT), a prominent polyphenol obtained from the olive tree, as a strategy to reduce the presence of harmful molecules, such as amines and nitrosamines, commonly found in cigarette smoke. In order to achieve this, we have developed a novel and enhanced approach by functionalizing cigarette filters with olive tree polyphenols, including HT and other phenolic compounds. The primary objective of this invention is to develop a cigarette filter that is enhanced with olive tree polyphenols, enabling the reduction of amines and nitrosamines within cigarette smoke and aerosol by establishing both strong and weak interactions with these compounds.

Filter

Cigarettes are made of tobacco rods or columns that, when ignited, produce both particulate matter and a vapor phase. Approximately 70 years ago, filters were introduced at the end of cigarette tobacco columns as a small device aimed at reducing the entry of harmful substances into the smoker's body, thereby mitigating health risks. As detailed in (8), Parliament (Benson and Hedges) introduced a premium-priced filtered cigarette brand in 1931, and Viceroy, originated in 1936, became the world's first cork-tipped filtered cigarette (source: https://tobaccotactics.org). During that period, cigarettes were generally around 70 mm in length and unfiltered, with most brands being similar in composition (9). Filters, among other functions, were designed to remove various smoke components. Filters made of filamentary or fibrous materials, such as cellulose acetate tow or paper, primarily address the particulate phase of tobacco smoke through mechanical means. However, these fibrous materials are less effective in eliminating volatile constituents present in the vapor phase, like aldehydes, hydrogen cyanide, amines, nitrosamines, and sulfides. To enhance the removal of vapor phase components, fibrous materials are often combined with adsorbents or absorbents. For instance, cigarette filters incorporate activated carbon and porous minerals like meerschaum, silica gel, cation-exchange resins, and anion-exchange resins (10,11). Various measures have been proposed to enhance the safety of cigarette filters, including the addition of anti-toxic flavoring agents (12). For instance, charcoal, due to its high specific surface area, serves as a potent adsorbent for vapor-phase constituents of tobacco smoke. Silica gels are also used, despite being generally considered as weak retentive adsorbents for vapor-phase tobacco smoke constituents. Weak basic anion-exchange resins with

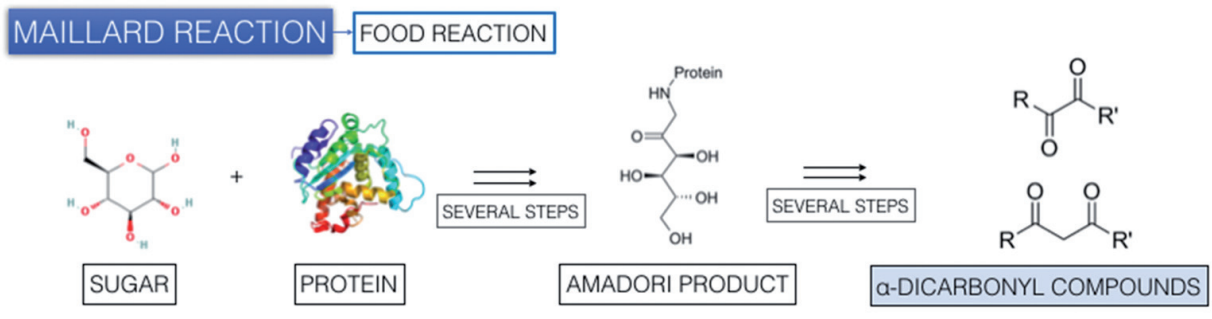

Fig. 1. A brief scheme representing the molecular mechanism of the Maillard reaction

porous structures prove effective in removing smoke acids and aldehydes, although their efficiency decreases during smoking, similar to carbon and porous minerals.

Despite perceptions that filters offer significant protection against the toxic compounds of smoke, such as nitrosamines, research has shown that filtered cigarettes are not substantially less harmful than unfiltered ones, for both smokers and passive smokers (source: https://tobaccotactics.org). Efforts to eliminate toxic compounds emitted during filtration have primarily focused on treating filters contaminated with toxicants, as detailed in ISO mainstream smoke measurements. These treatments have resulted in reduced yields of tar, nicotine, carbon monoxide, acrylonitrile, ammonia, aromatic amines, pyridine, quinolene, and hydrogen cyanide, along with increased yields of formaldehyde and isoprene (13).

Current research on available filters has concentrated on modifying these devices to enhance their filtering capacity and on recycling cigarette butts and trapped chemicals during filtration (14). The development of safer, more dependable, and reusable materials for filtering toxic compounds generated during combustion remains a challenge. This endeavor aims to reduce the health impacts associated with the cigarette industry's development of products that are still ineffective in fully safeguarding smokers against toxic compounds via filters. Studies and reports suggest that polyphenols can significantly reduce cytotoxicity, highlighting their protective role in lung epithelium (15).

Nitrosamines have been detected in various contexts, including food, beverages, air, cigarette smoke, cosmetics, and industrial environments. Tobacco-specific nitrosamines (TSNAs) are also prominent in tobacco and require comprehensive study and review (16-25). TSNAs are a group of carcinogens generated in tobacco smoke, originating from nicotine and related alkaloids during tobacco processing. Common TSNAs include NNN (N'-nitrosonornicotine), NNK ((4-methylnitrosamino)-1-(3-pyridyl)-1-butanone), NAB (N'-nitrosoanabasine), and NAT (N-nitrosoanatabine) (26). Research on hamster rats has shown that NNN and NNK induce tumors in the upper respiratory tract, and that NNK is the most potent carcinogen among TSNAs, inducing adenoma and adenocarcinoma in human lungs. Such harmful effects can be extrapolated to humans as well (26-31). Among nicotine-derived carcinogens, NNK is the most significant, and its secondary reduction produces NNAL, which has adverse health effects (32). Therefore, there is a pressing need for innovation to reduce the presence of such compounds through the functionalization of cigarettes.

Polyphenols

Numerous studies have consistently demonstrated that long-term consumption of polyphenols provides protective effects against a range of health conditions, including cancer, cardiovascular diseases, diabetes, osteoporosis, and neurodegenerative diseases (33-35). These findings strongly suggest that diets rich in polyphenols (such as those consisting of fruits, vegetables, tea, and coffee) offer substantial protection against the development of these debilitating illnesses.

Cardiovascular diseases, such as heart disease and stroke, often have a complex interplay between genetic factors (36-41) and lifestyle choices like smoking (42).

Research observations have indicated that the inhalation of tobacco, which contains nicotine, diminishes vascular PGL production and contributes to the onset of cardiovascular diseases (42).

Examining the chemical properties of polyphenols, it becomes evident that their interaction with electrophilic carbons in aldehydes or ketones occurs through an aromatic substitution reaction, a process inherently electrophilic in nature. A noteworthy attribute of polyphenols is their antioxidant property, which can be further enhanced by their ability to form metal chelates (43).

Despite numerous modifications in cigarette filters and the introduction of newer cigarettes with reduced nicotine and carbon monoxide content, there is no conclusive evidence to suggest a reduced risk of myocardial infarction for those who smoke these cigarettes compared to those who smoke the "classic" ones (44).

HT, also known as 4-(2-dihydroxyphenyl) ethanol, has emerged as one of the most potent natural antioxidants, ranking just below gallic acid (45). Numerous studies have highlighted the exceptional antioxidant and antimicrobial properties of HT, an ortho-diphenol known for its high bioactivity. Moreover, it has demonstrated significant beneficial effects on cardiovascular health and many other human diseases (45,46).

Polyphenol-enriched filter

An alternative approach to enhance the functionality of cigarette filters involves their functionalization using HT, a polyphenol extract derived from olive leaves. Polyphenols are known to reduce the production of reactive oxygen species by inhibiting oxidases, lowering superoxide production, inhibiting the formation of oxidized low-density lipoprotein (OxLDL), suppressing the proliferation and migration of vascular smooth muscle cells (VSMCs), reducing platelet aggregation, and improving mitochondrial oxidative stress (47). Moreover, HT possesses the capability to react with various toxic chemical compounds commonly found in cigarette filters.

An experiment conducted on rats (48) provided compelling evidence of the remarkable effectiveness of HT in reducing oxidative stress associated with passive cigarette smoke exposure.

Both in vitro and in vivo studies suggest that polyphenols hold significant potential as therapeutic agents, exhibiting cardioprotective, antimicrobial, anticancer, neuroprotective, and antidiabetic properties (49). Histological examinations have revealed that extracts from olives, thanks to their polyphenolic constituents, can effectively mitigate cigarette smoke-induced damage, including necrosis, pyknotic alterations, and congestion, in lung, hepatic, and renal tissues (50).

Aim of work

Our patent introduces an innovative approach in this domain, presenting a filter functionalized with olive tree polyphenols, with a particular emphasis on HT. The central goal of this invention is to address the issue of amines and

nitrosamines generated during cigarette combustion, using the reactivity of olive tree polyphenols to establish both strong and weak interactions with these compounds.

Furthermore, the polyphenols introduced through this filter are capable of reducing the production of reactive oxygen species through mechanisms such as inhibiting oxidases, lowering superoxide production, inhibiting OxLDL formation, suppressing VSMC proliferation and migration, reducing platelet aggregation, and enhancing mitochondrial oxidative stress (47).

Another critical aspect of this invention is the proposal of a method or approach for a novel filter, enriched with olive tree polyphenols, primarily HT, which can be sourced from olive leaves or fruit. This specialized filter is explicitly designed to counteract the formation of amines and nitrosamines during cigarette combustion. In essence, our innovation seeks to provide a safer and more effective alternative to traditional cigarette filters, aiming to reduce the harmful compounds generated during smoking.

Materials and Methods

Hydroxytyrosol extraction

As previously mentioned, HT, a valuable polyphenol with numerous beneficial properties, is a key component of our innovation, can be efficiently extracted from olive trees using contemporary and highly effective techniques, such as employing Irred-Irad® as a pretreatment method (51). This cutting-edge approach enhances the extraction process, ensuring optimal yield.

HT is derived from the hydrolysis of oleuropein, a compound that develops naturally during the maturation of olives. This process leads to the formation of oleuropein aglycone, HT, and elenolic acid (52). Furthermore, an alternative way of obtaining HT is from olive mill waste, showcasing the versatility and sustainable sourcing possibilities of this natural molecule (53,54). This diverse range of extraction methods underscores the potential for HT to serve as a valuable component in our innovative filter design.

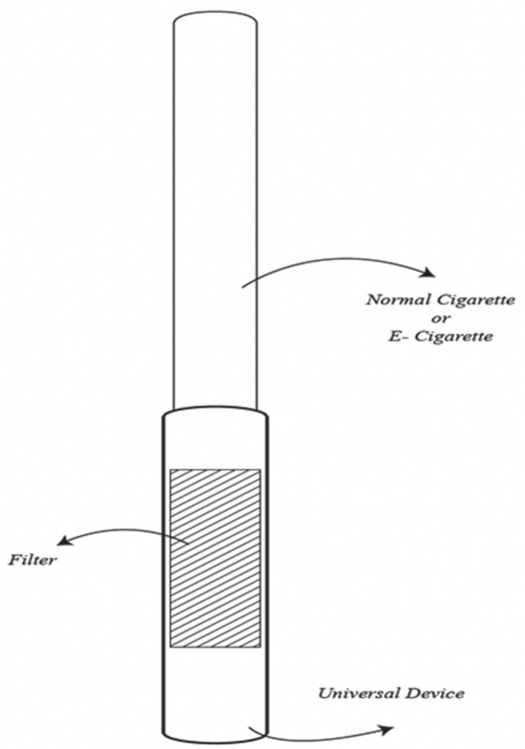

Fig. 3. Another view of the claimed assembly per preferred embodiments of the filter.

Cellulose acetate for making filters

Cellulose acetate filters are a widely employed method for creating filters in cigarettes, given their prevalence in the industry. It is worth delving into the research, detailed in reference (55), to better understand the process involved. This research used various materials and equipment, including fibrous material, staples, calcium chloride, acetic acid as chemical reagents, stainless steel tubes,

Teflon capsules, glass joints, heating tubes, heater coils, ground-glass joints, glass calibration ampoules, a spectro-

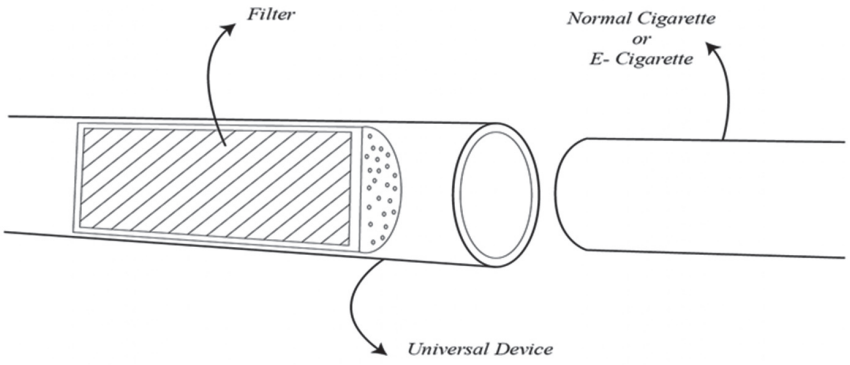

Fig. 2. Exploded view of the claimed assembly.

meter, commercial-grade triacetate, secondary acetate fibers, and titration equipment. The method outlined in (56) begins by cutting the fibrous material into pieces, compacting the staples, and placing them in a desiccator containing calcium chloride, to ensure dryness. A stainless-steel tube is used to secure one end of a capsule, while the other end of the tube is equipped with a glass joint. A controlled airflow of approximately 17.5 ml/sec is drawn into the system to assess the sorption capacity of the fibrous material, with the other end of the capsule serving to measure air resistance. After elution with solvents, the proportion of the substance that remains in the filter paper is calculated through spectrophotometric or titrimetric analysis. To prepare the fibers, they are moistened by passing them through an aqueous bath of acetic acid (Figure 2, and Figure 3).

Filter nanocoating

The subsequent phase of our research focuses on functional nanocoating applied to the filter surface. This research, as described in Sadabad et al. (2019), explores the application of functional nanocoating in the production of commercial cigarette filters. The study employs a range of reagents and materials, including pyrogallol (PG), gallic acid (GA), pyrocatechol (Ctl), epigallocatechin gallate (EGCG), tannic acid (TA), catechin (Ctn), hydroxyhydroquinone (HHQ), caffeic acid (CA), morin, sodium ascorbate (SA), glutathione (GSH), and uric acid (UA) as natural antioxidants. These phenolic compounds are central to the study, due to their potential in retarding the oxidation kinetics. In the investigation (43), focused on reducing toxic carbonyl species in e-cigarettes, the in vitro trapping properties of Reactive Carbonyl Species (RCS) by gallic acid, HT, and epigallocatechin were explored based on their chemical structures and ability to form adducts with glyoxal and methylglyoxal. The study also involved the use of glycerol and propylene glycol, as well as various chemical reagents, such as 2,4-dinitrophenylhydrazine (DNPH) and the most commonly employed polyphenol, including gallic acid, HT, and epigallocatechin gallate, among others. The experimental process included the preparation of e-liquid formulations containing different concentrations of epigallocatechin, HT, and gallic acid, which were then subjected to vaping using a specialized device. Subsequent analysis involved High-performance liquid chromatography-ultraviolet (HLPC-UV) and Liquid chromatography-tandem mass spectrometry (HPLC-ESI-MS/MS) to evaluate carbonyl and dicarbonyl compounds. The study also encompassed cell culture and cytotoxicity assessments.

The grafting process involved the use of a glass Petri dish and cellulose samples, which were treated with laccase, catechin, and sodium tartrate buffer under controlled conditions. Cellulose samples were washed and air-dried as part of the process (57). As for the sample preparation in the subsequent embodiment, commercial cigarette filters were utilized, with two distinct types of filters included:

- Polyphenol Treated Filter (PT): The filter extracted from a commercial cigarette was treated with a 500 µL solution of olive polyphenols, titrated to HT;
- Negative Control Filter (NC): The filter from a commercial cigarette remained untreated.

Both filters were integrated into a vacuum system, connected to an aerosol nebulizer device. The smoke emitted from PT and NC filters was analyzed using the Secondary Electrospray (SESI) technique, enabling the identification and quantification of volatile organic compounds in the smoke and aerosol.

Key parameters for the SESI technique included an aspiration value of 1.2 L/min, a capillary voltage of 3 kV, a nebulizer gas flow rate of 5 µL/min, and a curtain gas flow rate of 1.2 L/min. The solution used for spray production comprised H_2O/CH_3OH (water/methanol), with 0.1% formic acid. Data analysis was conducted using the SANIST-ORBIT platform.

Results

In the following section, we will present the key findings from the study conducted to assess the effectiveness of a cigarette filter enriched with polyphenols. The primary objective of this research was to investigate whether the incorporation of polyphenols, renowned for their antioxidant and health-protective properties, could significantly alter the composition and toxicological impact of chemicals in cigarette smoke. A comprehensive series of tests were meticulously conducted, comparing cigarette smoke generated through traditional filters with smoke produced using polyphenol-containing filters. The results are expected to yield valuable insights into the influence of this innovative technology on the health risks associated with cigarette consumption.

Table 1 presents compelling evidence of a substantial reduction in signal intensity for various molecules, including :

Formaldehyde: The spectrophotometer recorded an intensity of 1154 AU for the control filter, while for the filter enriched with polyphenols, it detected a concentration of 342.

Acetone: The spectrophotometer read an intensity of 1254 AU for the control filter, whereas for the polyphenol-enriched filter, it registered a concentration of 331 AU.

Acrolein: The spectrophotometer measured an intensity of 1698 AU for the control filter, whereas for the polyphenol filter, it identified a concentration of 478 AU.

Aniline: The spectrophotometer noted an intensity of 2144 AU for the control filter, while for the polyphenol filter, it observed a concentration of 564 AU.

O-Tuluidine: The spectrophotometer detected an intensity of 1876 AU for the control filter, but for the polyphenol filter, it reported a concentration of 531 AU.

2,4,6-Trimethyline: The spectrophotometer recorded an intensity of 1874 AU for the control filter, whereas for the polyphenol filter, it found a concentration of 598 AU.

Anisidine: The spectrophotometer measured an intensity of 1977 AU for the control filter, but for the polyphenol filter, it detected a concentration of 502 AU.

N-nitrosonornicotine: The spectrophotometer read an intensity of 2030 AU for the control filter, while for the polyphenol filter, it determined a concentration of 1125 AU.

Table 1. The most significant molecules aerosol in the PT filter compared with the NC filter.

Molecules	m/z	Ion Detected	Spectral Intensities NC (AU)	Spectral Intensities PT (AU)
Formaldehyde	67	$[M+H+2H_2O]^+$	1154	342
Acetone	59	$[M+H]^+$	1254	331
Acrolein	75	$[M+H+H_2O]^+$	1698	478
Aniline	94	$[M+H]^+$	2144	564
O-Toluidine	108	$[M+H]^+$	1876	531
2,4,6 – Trimethylaniline	136	$[M+H]^+$	1874	598
Anisidine	124	$[M+H]^+$	1977	502
N'-nitrosonornicotine	178	$[M+H]^+$	2030	1125

Based on the highlighted data, it is reasonable to hypothesize that polyphenols, such as HT, effectively function as filters for highly harmful molecules in cigarette smoke. Future investigations will be essential to explore how polyphenols, such as HT, can be harnessed to capture other equally significant toxic compounds that are detrimental to human health, such as amines and nitrosamines.

Comparative analysis of plots

Spectra of the detected compounds are represented in Figures S1-S8.

In the first spectrum of each molecule:

The black spectrum refers to the compounds detected in cigarette smoke with an NC filter.

The red spectrum refers to the compounds detected in the cigarette smoke with the PT filter.

Discussion

A thorough series of tests was meticulously carried out to compare cigarette smoke produced through traditional filters with that generated using polyphenol-enriched filters. The findings presented in Table 1 demonstrate a substantial reduction in the signal intensity of various molecules when the polyphenol-enriched filter, referred to as the PT filter, was utilized. Notably, Formaldehyde exhibited a reduction of 71%, Acetone showed a reduction of 74%, Acrolein displayed a reduction of 72%, Aniline exhibited a reduction of 74%, O-Tuluidine displayed a reduction of 72%, 2,4,6-Trimethyline showed a reduction of 68%, Anisidine exhibited a reduction of 75%, and N-nitrosonornicotine revealed a reduction of 45%. These results strongly indicate that the polyphenol filter effectively mitigates the presence of some of the most prevalent and toxic compounds in cigarette smoke compared with the traditional NC filter.

However, it is important to note that our study primarily focused on a select group of toxic compounds. Future investigations will be crucial in unraveling the potential of polyphenols, such as HT, in capturing other equally significant toxic compounds that pose a threat to human health, such as amines and nitrosamines. This promising avenue of research holds the potential to contribute significantly to harm reduction strategies related to smoking and tobacco consumption.

Conclusions

Our study aimed to investigate the chemical functionalization of cigarette filters with olive tree polyphenols, particularly HT, and to evaluate their ability to remove carcinogens from cigarette smoke and aerosol. To determine the filtration efficacy of the developed filter, we employed an automated Stain Pattern technique, which allows for non-intrusive measurement of behavioral vent blocking (58). Our objective was to modify the surface of cigarette filters to reduce the presence of nitrosamines in cigarette smoke, a byproduct of combustion. We sought to establish a mechanism to create a highly efficient filter.

Based on our comprehensive study, it is clear that further research on the functionalization of cigarette filters using polyphenols like HT, which can effectively trap and mitigate the formation of toxic chemicals such as nitrosamines (e.g., N'-nitrosonornicotine, 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone, N'-nitrosoanatabine, and N'-nitrosoanabacine), and other chemicals (formaldehyde, acetone, acrolein, aniline, O-Tuluidine, 2,4,6-Trimethyline, and anisidine) during cigarette smoking, is necessary. Functionalizing cigarette filters with a high filtering efficiency and a capture factor ($f=2.9\times10^3$) for two layers, as described in the article (59), can revolutionize the use of filters in commercial cigarettes. This innovation can significantly reduce consumers' exposure to various toxic chemicals, including nitrosamines when HT is incorporated into commercial filters.

Our tests have shown that polyphenols, and particularly HT, have the capacity to effectively remove toxic chemicals like nitrosamines from cigarette smoke. Functionalizing cigarette filters with HT represents a potentially revolutionary invention for mitigating the presence of harmful compounds in cigarette smoke. However, further in-depth research is imperative to assess the protective actions of HT-functionalized cigarettes and to determine if this innovation can indeed safeguard consumers' health effectively.

Acknowledgements

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Conflicts of interest statement

Authors declare no conflict of interest.

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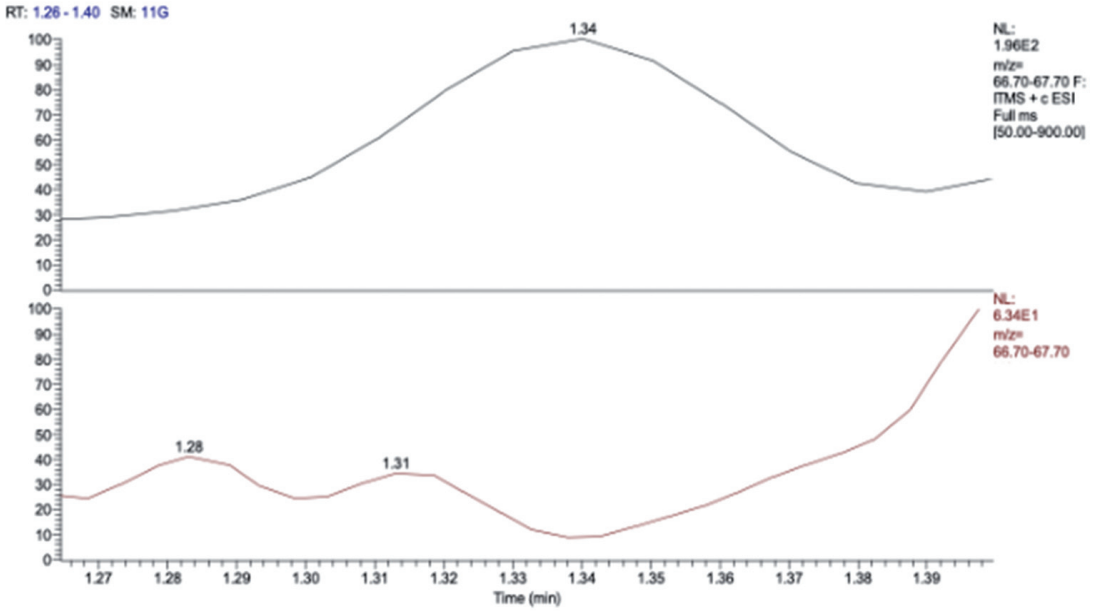
SUPPLEMENTARY MATERIALS

Figure S1. Spectra of formaldehyde.



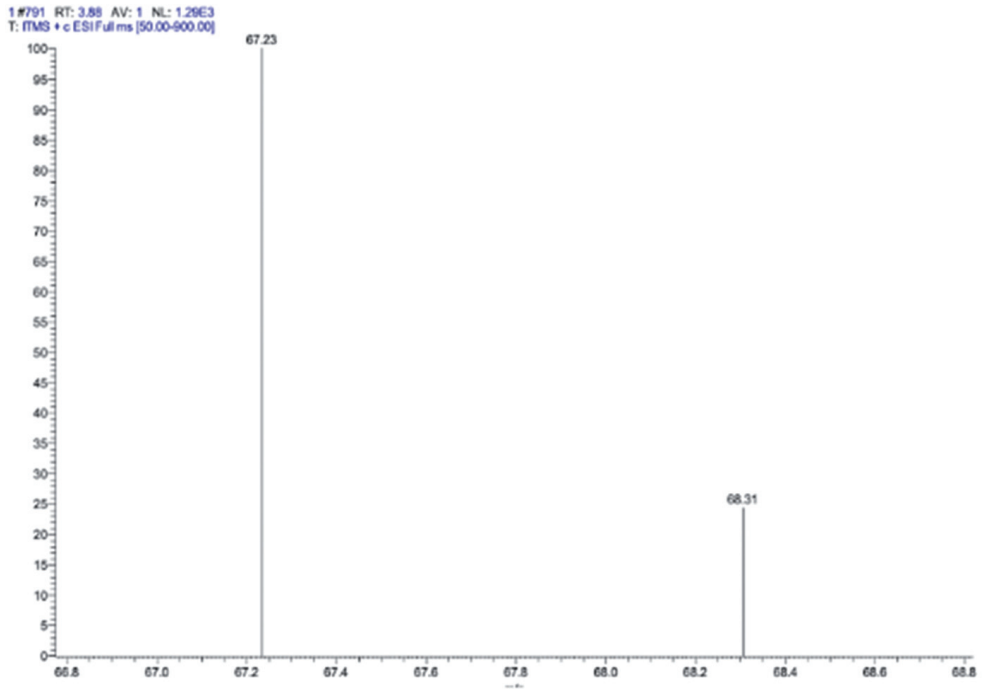
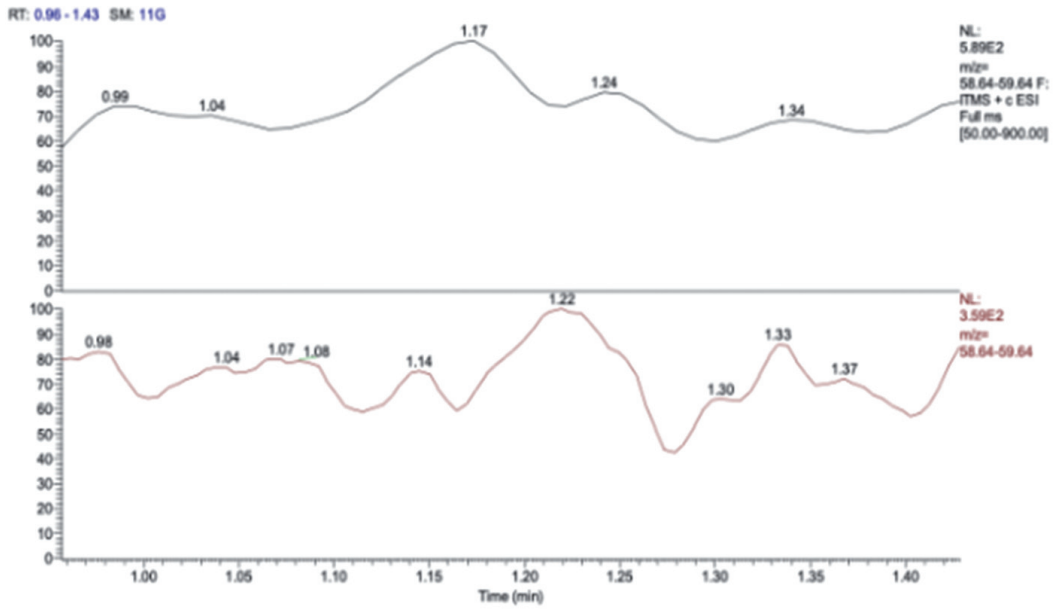


Figure S2. Spectra of Acetone



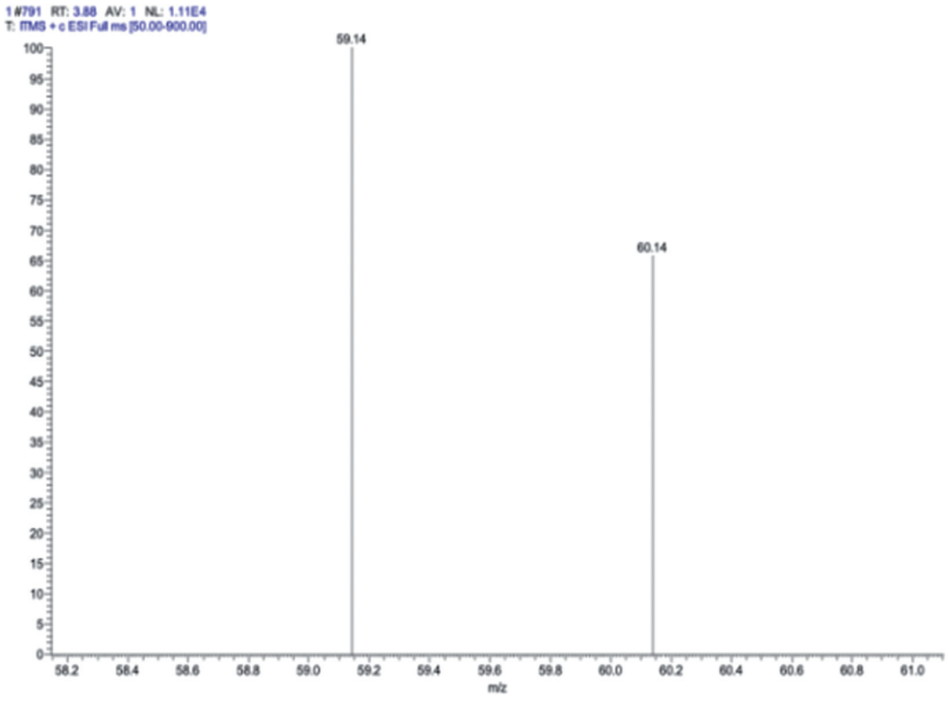
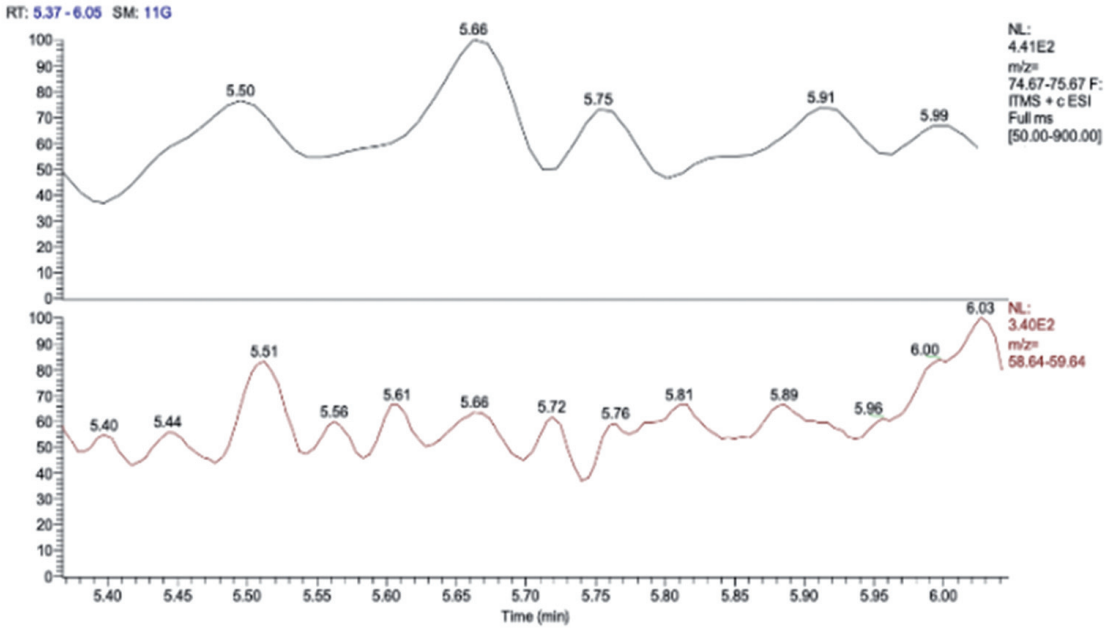


Figure S3. Spectra of Acrolein



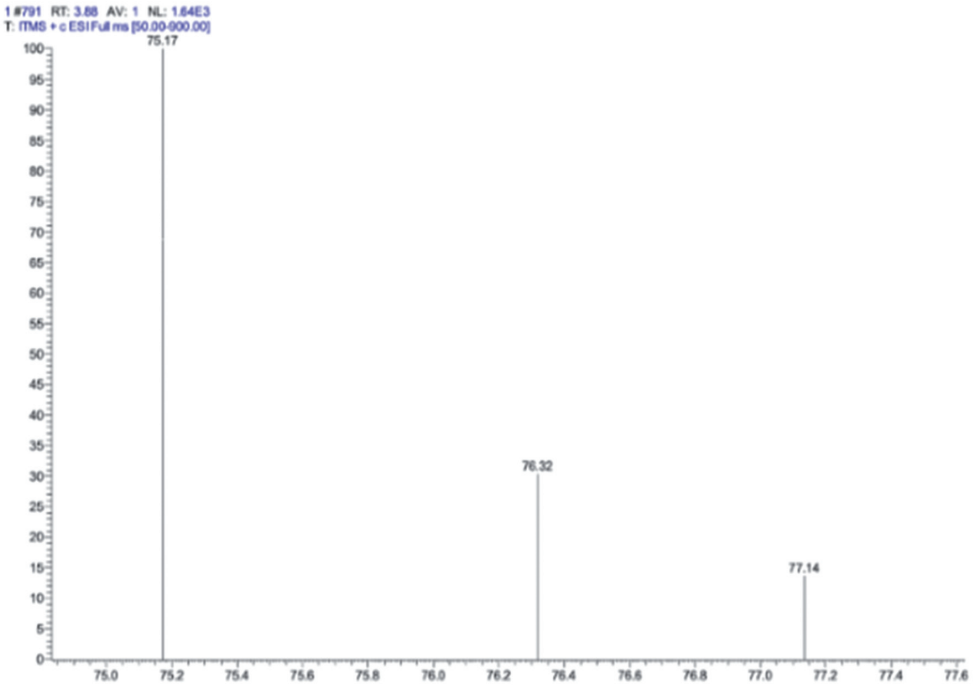
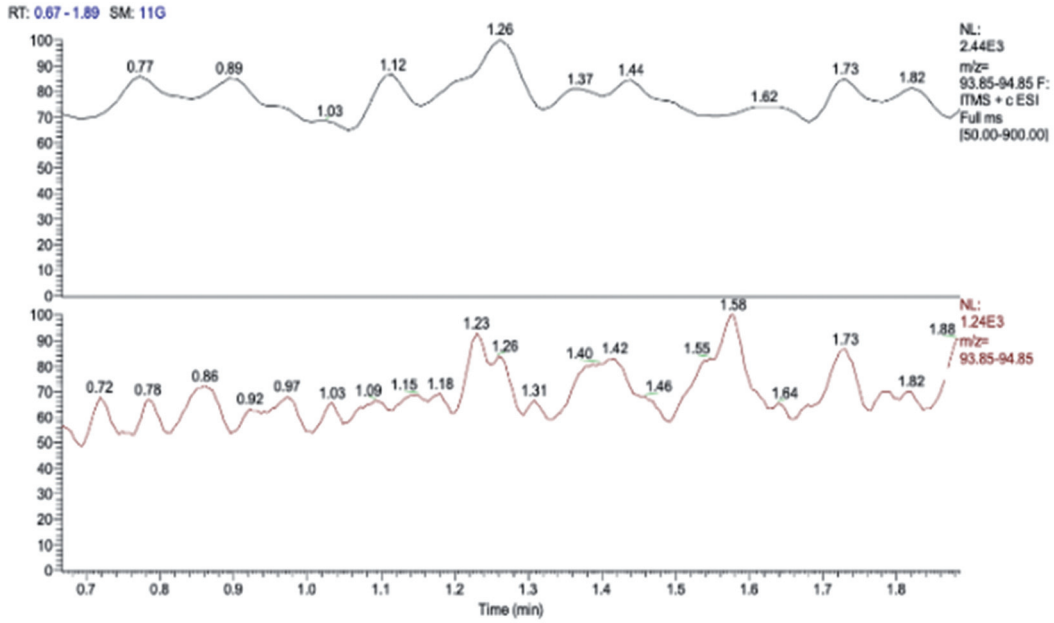


Figure S4. Spectra of Aniline



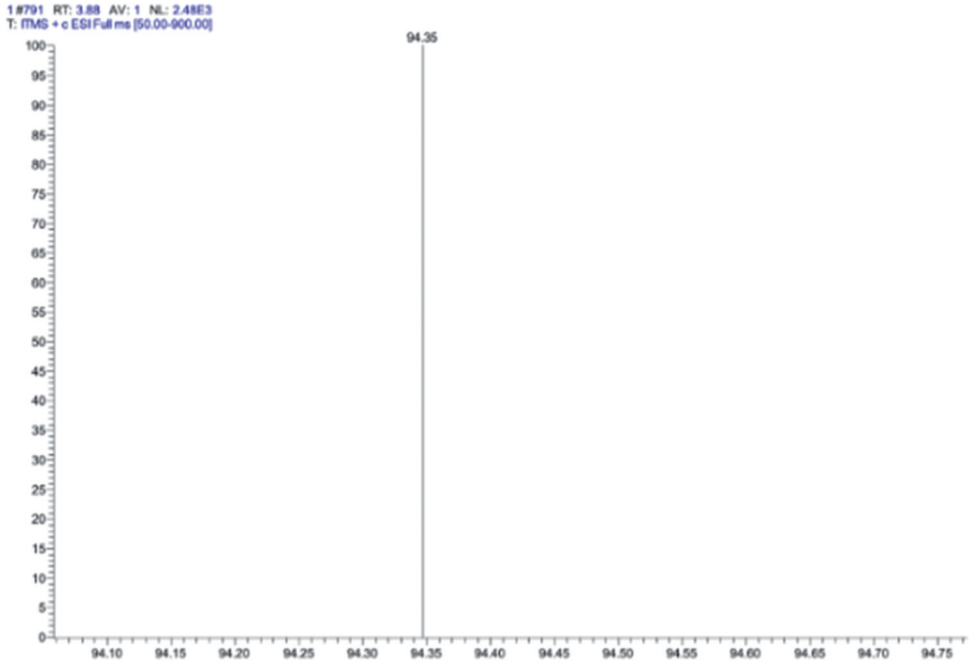


Figure S5. Spectra of O-Toluidine

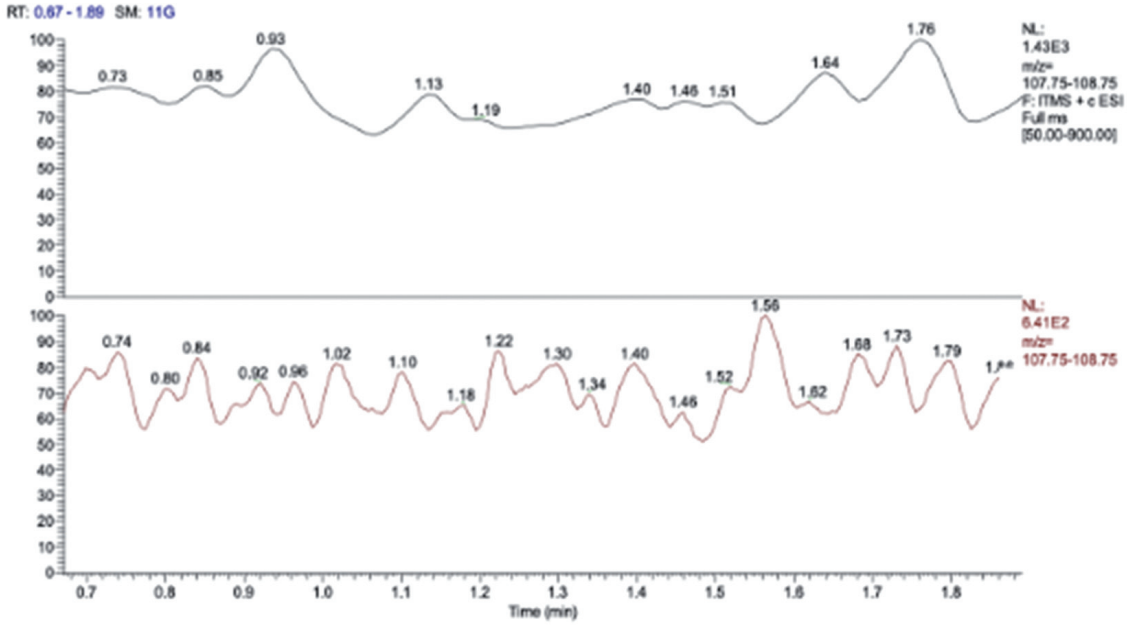
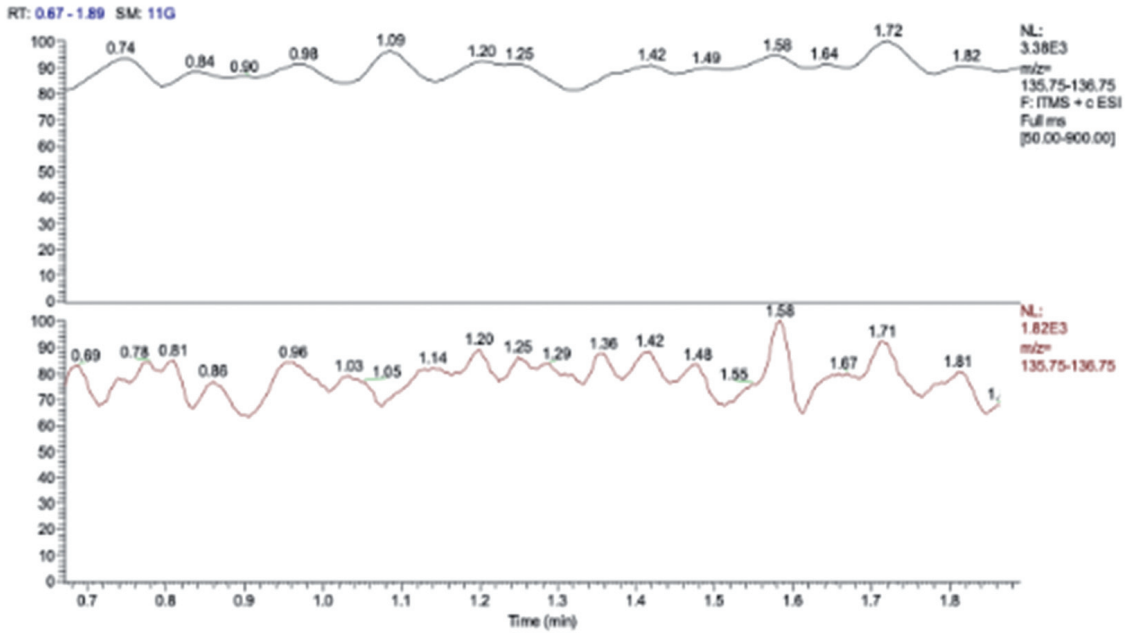


Figure S6. Spectra of 2,4,6-Trimethylaniline



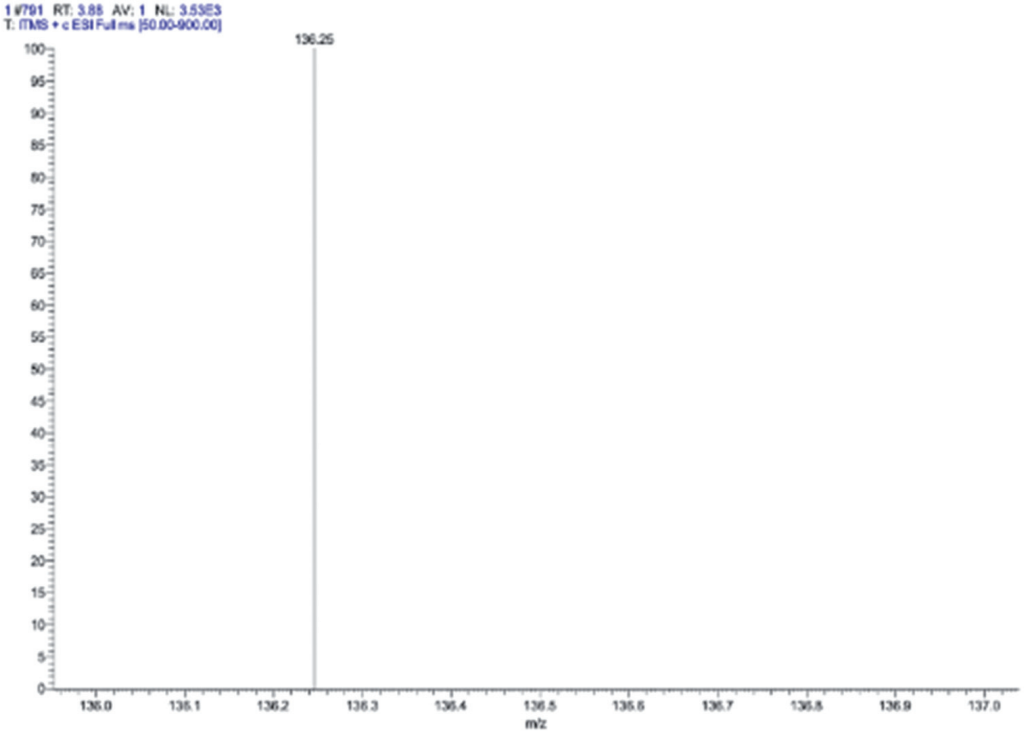
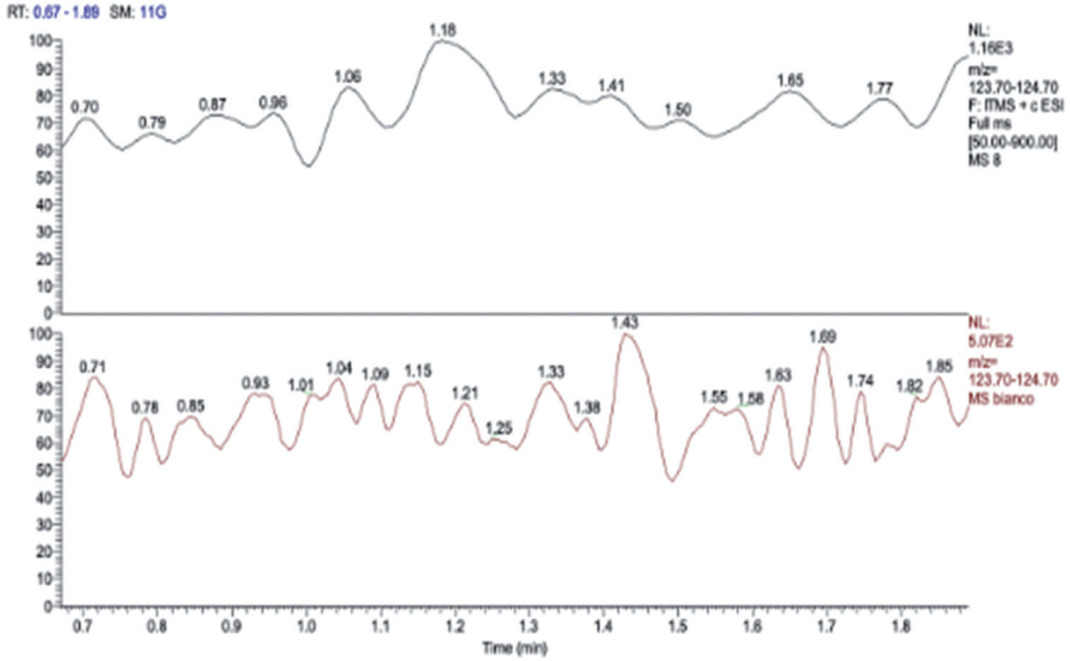


Figure S7. Spectra of Anisidine



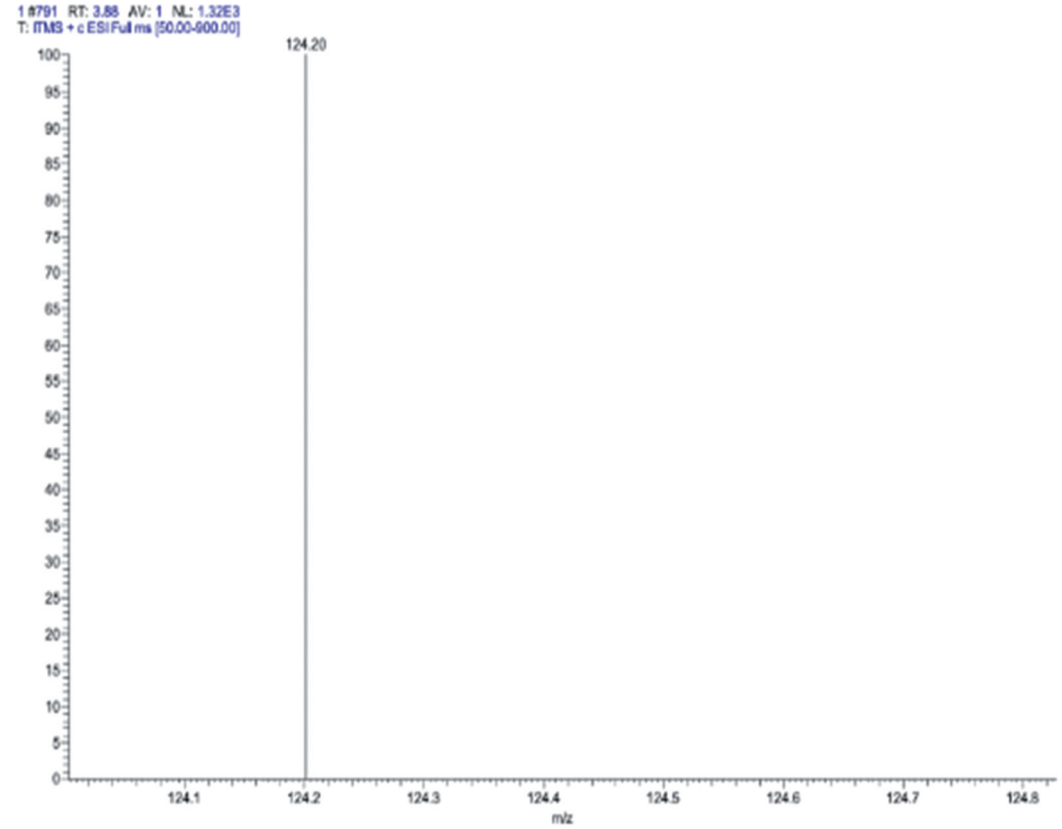
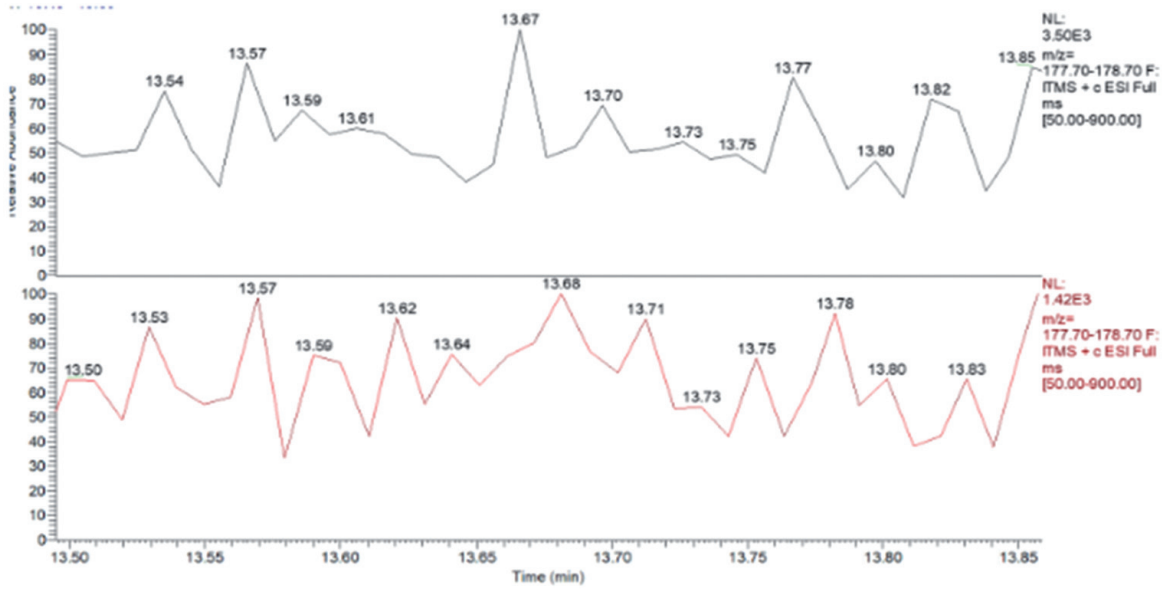
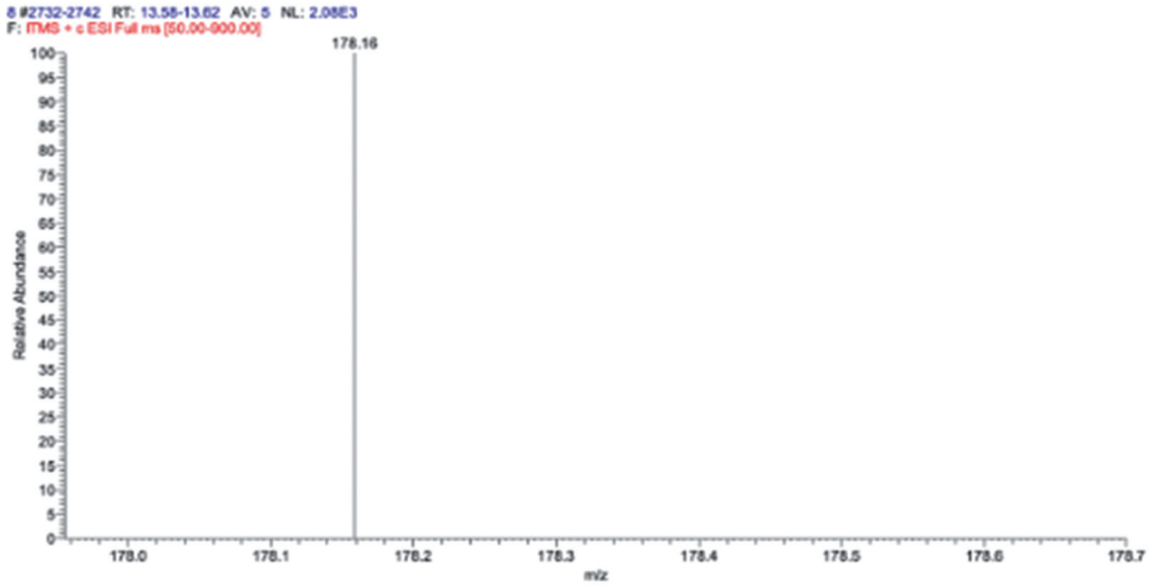


Figure S8. Spectra of N'-nitrosonornicotine





Review *Clin Ter 2023; 174 Suppl. 2 (6):142-148 doi: 10.7417/CT.2023.2480*

The Role of Olive Tree Polyphenols in the Prevention of COVID-19: A Scoping Review, part 1

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Abstract

The global COVID-19 outbreak, started in December 2019, resulted in severe financial losses and extraordinary health crises. Finding a potent and secure medication candidate to treat SARS-CoV-2 infection and its symptoms is still an urgent global need. After reviewing previous studies, olive leaves, being rich in polyphenolic compounds (a large class of bioactive substances naturally found in plants), were proposed as a viable co-therapy supplement to treat and improve clinical symptoms in COVID-19 patients. It has long been known that olive tree polyphenols—such as oleuropein, hydroxytyrosol, verbascoside, as well as triterpenoids like maslinic, ursolic, and oleanolic acids—have anti-inflammatory and multitarget antiviral effects on several virus families, and they could be one of the reasons of the beneficial effects of the Mediterranean diet against COVID-19. Thus, olive tree polyphenols were tested in silico and in vitro for preventing SARS-CoV-2 infection, claiming that they have beneficial effects. Nevertheless, there is still a small number of research studies on this topic. The aim of this scoping review is to provide more information and offer an opinion on the feasibility of using olive tree polyphenols as a springboard for the creation of innovative natural remedies against this viral illness, ultimately planning future relevant studies. ***Clin Ter 2023; 174 Suppl. 2 (6):142-148 doi: 10.7417/CT.2023.2480***

Key words: *SARS-CoV2, COVID-19 pandemic, polyphenols, antiviral, olive tree*

Introduction

The COVID-19 pandemic started in December 2019, and it caused both economical and sanitary crisis worldwide. As of August 2022, the World Health Organization estimates that there have been almost 600 million COVID-19 cases and more than 6 million fatalities globally (1). The development of therapeutic leads that combat COVID-19 infection using various methods—including in silico, in vitro, in vivo, and clinical trials—is now a global trend. However, FDA has only recently authorized just a few medicines for treating and preventing COVID-19 (2). As a result, it is imperative to create antiviral drugs that can manage the infection.

SARS-CoV-2 infect the respiratory tract, causing also a systemic infection, which displays a significant immune response, leading to multiorgan dysfunction. This is in contrast to different coronaviruses, which cause only respiratory illnesses (3). Indeed, the primary symptoms of COVID-19 disease are fever, headache, pneumonia, and loss of smell and taste (4,5). Symptoms differ from patient to patient, based on viral load and virus strain (5).

Hyper-inflammation, brought on by the related cytokine storm, particularly IL-6, leads to acute respiratory distress syndrome, the main cause of mortality in COVID-19 patients (6,7). Furthermore, COVID-19 causes disseminated intravascular coagulation, a hazardous side effect; indeed, numerous studies revealed a connection between COVID-19 and an increase in thrombotic events (8,9). At the moment, COVID-19 patients are being treated with a variety of medications, including anti-SARS-CoV-2 monoclonal antibodies, antivirals, immunomodulators, and antithrombotic drugs. These drugs are able to manage the symptoms of COVID-19, but no treatment is able to effectively cure the disease (7,8).

Plant-based natural products have been proposed for treatment of SARS-CoV-2 infection. Indeed, natural products have historically been proposed for treatment of respiratory diseases, and a variety of natural molecules derived from

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plants are now studied for their antiviral properties (10). One of the most common natural products used in the nutraceutical industry for treating many diseases are olives (*Olea europaea L.*, family Oleaceae). The olive tree originated in Asia, but it is now cultivated across the Mediterranean region, in Iran, and in Northern Africa. In the nations where COVID-19 infection is endemic, olive trees are common and are utilized medically as antiviral drugs (11), so olive leaves and their extract could be used as a phytotherapy for COVID-19 treatment (12). Indeed, they contain polyphenolic components such as oleuropein, hydroxytyrosol (HT), verbascoside, apigenin-7-O-glucoside, which have several biological activities, including antioxidant, antihypertensive, anti-hypercholesterol activity (11–16).

Polyphenols are one of the biggest and most investigated family of bioactive substances found in many plants. They are formed as secondary metabolites and have anti-cancer radiation, anti-pathogen, and anti-oxidative stress properties (17). According to their structural definition, polyphenols are compounds that have one or more phenolic rings with hydroxyl groups. Polyphenols can be divided into flavonoids (which include stilbenes or lignans), phenolic acids, polyphenolic amides, and other (such as anthocyanins, flavones, flavonols, flavonones, flavonols, and flavan-3-ols) (18). Evidence has demonstrated polyphenols have several beneficial properties, among which anti-inflammatory, antioxidant and antiviral effects (19–24). Numerous studies have specifically shown that polyphenols are effective against a variety of pathogens, such as the herpes simplex virus (19,20), influenza virus (21), and other viruses causing infections of the respiratory tract (22,23). In this context, resveratrol was extensively studied because of it can (i) disregulate viral replication by reducing the expression of immediate-early virus proteins; (ii) inhibit the NFkB signaling cascade; and (iii) activate the AMPK/Sirt1 pathway (20). Moreover, elenolic acid, a metabolite of olive polyphenols, acts as an antiviral in the form of calcium enolate, inhibiting reverse transcriptases (25,26).

Polyphenols were proposed for the treatment of COVID-19 mainly because of their antiviral and immunomodulatory activities (12,27). The goal of this scoping review is to highlight some encouraging data about olive tree polyphenols' possible anti-COVID19 action, which might spur research into the creation of new approaches to fight the SARS-CoV-2 pandemic.

Materials and Methods

Literature Scoping Review and Inclusion Criteria

The scoping review section followed PRISMA guidelines for scoping reviews (28). Our search included original and review articles focusing on SARS-CoV-2 and the effects of olive polyphenols, HT, oleuropein, oleochantal, oleanolic acid, and olive leaf.

The articles employed had to be written in English and published in the period 2019-2022. Congress abstracts, manuscripts not written in English and studies not relevant to the topic of the present manuscript were excluded. Articles previously published by the MAGI laboratories or by any of the authors of this paper were not considered for this scoping review.

Literature Search

PubMed database was searched to retrieve articles published from 2019 to 2022 that satisfied the inclusion criteria. The search keywords were: (olive polyphenols OR hydroxytyrosol OR oleuropein OR oleochantal OR oleanolic acid OR olive leaf) AND (SARS-CoV-2 OR COVID-19 OR long COVID OR post COVID). In addition, the reference lists of the papers found were scanned manually to find further relevant papers.

Study Selection

All the resulting articles were assessed independently for eligibility by authors who evaluated titles and abstracts according to the above inclusion criteria. Once a paper was found eligible, its references were screened to find new papers.

Results

47 papers published from 2019 to 2022 were found, based on the literature review search criteria. Only 44 pertained to the topic of the current review and were read completely. Another 10 papers were included after reading the references of the articles. Finally, 28 studies, considered to be the most relevant, were discussed in the manuscript.

The following sections will highlight the main findings of the included articles. Indeed, several studies have been published on the antiviral, anti-inflammatory, immunomodulatory, and antithrombotic effects of olive leaf extract, both in its entirety and considering only its components. Moreover, possible advantages of additional in vivo studies or clinical trials studying the effects of olive leaf-based natural supplements will be discussed.

In Silico Antiviral Studies

Several in silico investigations suggest the efficacy of olive leaf metabolites against SARS-CoV-2. Many viral proteases, the spike protein, and the ACE-2 receptor were studied for their binding affinity against olive leaf polyphenols, among which: oleuropein, HT, oleanolic acid, maslinic acid, ursolic acid, rutin, luteolin, luteolin-7-O-glucoside, quercetin, kaempferol, verbascoside, caffeic acid, gallic acid, ellagic acid, epicatechin (29–35). Many of these studies revealed high binding affinity of polyphenols to viral proteins.

The study by Thangavel et al. (36) found potential secoiridoids that can block SARS-CoV-2 entrance, replication, and related hyperinflammatory reactions thanks to molecular docking and molecular dynamics-assisted virtual search of OliveNetTM directory. OliveNetTM is an ongoing database of phytochemicals derived from various olive tree components (Oleaceae). The Mediterranean and Arabian diets are inextricably linked to olive oil and to the phenolic-rich olive fruits that are recognized for their health advantages. The

study also evaluated 78 secoiridoids, showing that two main secoiroids, Nüzhenide oleoside and Demethyloleuropein have an inhibitory effect on the spike protein and SARS-CoV-2 main protease M^{pro}. Moreover, Nüzhenide oleoside and other secoiroids identified by molecular docking showed an anti-inflammatory potential, because of an inhibitory effect to IL1R, IL6R, and TNFR1 (36).

Another study by Alhadrami et al. (37) investigated the inhibitory potential of betulinic acid and its anti-SARS-CoV-2 effect acting on its main protease. Betulinic acid and three other triterpene congeners present in olive leaves (ursolic acid, maslinic acid, and betulin), inhibit M^{pro} (37).

A study by Vijayan et al. (38) examined the role of thymopentin and oleuropein, phenolic compounds found in olive tree leaves, as potent inhibitors for SARS-CoV-2. They screened a natural product database for compounds against the viral protein NSP15, and found that thymopentin and oleuropein from olive tree bind to NSP15 forming a stable complex with high binding energy (38).

Gosh et al. (39) screened natural compounds from olives as possible inhibitors of protein tyrosine kinase (PTK). PTK has a role in both cancer progression and coronavirus infection. Indeed, overexpression of PTK enhances viral infectivity, and the use of tyrosine kinase inhibitors reduces the infection length. They virtually screened 161 natural compounds from olives and employed multilinear regression QSAR-based model and docking. The QSAR-based virtual screening successfully identified several possible inhibitors, and the best-docked ones were further investigated, revealing the high potential of many natural molecules derived from olives in PTK inhibition.

In Vitro Antiviral Studies

A small number of in vitro research examined the direct effects of polyphenols from olive tree extract on coronaviruses, in addition to the general mechanisms of action outlined against different viruses. HT found in olive leaves is demonstrated to have antiviral activity against SARS-CoV-2. Indeed, HT targets the spike proteins the viral genome (40). In a different investigation, luteolin had similar results, reducing SARS-CoV-2 infection in vitro (41,42). Furthermore, kaempferol prevented SARS-CoV-2 multiplication in culture with high percentage of inhibition (43).

A study by Takeda et al. (40) examined the SARS-CoV-2-inactivating activity of HT-rich aqueous olive pulp extract (HIDROX®) in vitro. They have demonstrated that the HIDROX® solution has anti-SARS-CoV-2 effects that are time- and concentration-dependent, and higher than pure HT. Moreover, they analyzed the mechanism of action of HIDROX® and HT, demonstrating that both structurally change SARS-CoV-2, altering the spike protein. As a result, the cream containing HIDROX® can be used topically as a hand lotion to combat viruses, helping to make SARS-CoV-2 prevention strategies more effective.

Clinical Antiviral Studies

Bezmialem Vakif University conducted a clinical study (44) investigating the possible effects of effects of drinking Olive Leaf Tea (OLT) in influencing the immune response and COVID-19. The study enrolled 249 workers, 168 of which were OLT drinkers. The results showed higher values of NK cells, NKT cells, total NK cells, and serum IFN-γand IL-2 levels in OLT drinkers as compared to the non-drinkers. Thus, they proved that OLT drinkers have an altered immune defense mechanisms. Moreover, specific COVID-19 IgG levels were found in 60% of OLT drinkers and in 38% of OLT non-drinkers, although both groups reported no history of COVID-19. The authors concluded that drinking OLT could help in fighting COVID-19 by increasing the innate immune response (44).

Another clinical trial study at Fayoum University Hospital in Egypt (45) will enroll RT-PCR confirmed-COVID-19 adult patients showing mild to moderate disease, excluding patients with multi-organ failure, ventilator support, and chronic diseases. Patients will receive, along with standard care, capsules containing oleuropein from olive leaves or placebo up to 10 days. The controlled outcomes include symptom alleviation, virus clearance, improvement of blood biomarkers and reduction of mortality (45).

The Spanish Hospital of Jean will begin a clinical trial to test a dietary supplement made of olive polyphenols and flavonoids for preventing the progression of COVID-19. They will recruit COVID-19 positive patients over 60 years old, and they will evaluate how many patients will progress from mild to severe symptoms. They will measure several parameters, among which cytokines, ferritin, D-Dimer, antioxidant markers, and thrombogenicity (46).

Role of the Mediterranean Diet in COVID Prevention

Obesity, type 2 diabetes, and hypertension are all common COVID-19 risk factors. They are all related to nutrition, thus implying that healthy diets may reduce COVID-19-related outcomes and SARS-CoV-2 infection.

Green at al. (47) studied the association between Mediterranean diet adherence and COVID-19, based on the influence of nutrition on immune function. Moreover, the Mediterranean diet reduces the risk to develop several chronic diseases that are comorbidities in COVID-19 patients. In this regard, they also investigated the relationship between regional adherence to a Mediterranean diet and COVID-19 cases and fatalities. They found that, in 17 areas of Spain, adherence to the Mediterranean diet was negatively linked with both COVID-19, and that the association persisted after adjusting for well-being characteristics. They also saw a negative correlation between COVID-19-related fatalities and adherence to the Mediterranean diet across 23 nations. The Mediterranean diet's anti-inflammatory effects, which are probably caused by the diet's high content of polyphenols from olive oil, could be the biological explanation for their findings.

Ferro et al. (48) showed how to assess the potential benefits of a Mediterranean diet in reducing the risk of coronavirus illness. Moreover, they also evaluated the possible anti-COVID-19 action of many vitamins, minerals, fatty acids, and phytochemicals found in olive tree leaves extracts. They examined how the Mediterranean diet is beneficial for the immune system and inflammation, showing that it may protect against SARS-CoV-2.

Perez-Araluce et al. (49) presented the first epidemiological research that has linked a dietary pattern to COVID-

19. Their findings suggest that greater adherence to the Mediterranean diet may be linked to a reduced chance of developing COVID-19 in the future. They discovered that people following the Mediterranean diet have a risk reduction of more than 60%. Despite the promising results, one of the major limitations of this study is the lack of data on the exposure to the virus that each individual may have had, also depending on how strictly they adhered to other non-pharmacological preventative measures.

Finally, Kim et al. (50) examined the effects of plant-based and pescatarian diets on COVID-19 severity. They recruited people from six countries and had them complete a survey on dietary information and COVID-19 outcomes. The results showed that vegetarian or pescatarian diets were linked to a decreased risk of moderate-to-severe COVID-19 in six different nations. These eating habits could be taken into account as potential COVID-19 protection.

Reviews on Polyphenols for COVID19 Treatment

Santos et al. (51)'s research examined the anti-inflammatory effect of a polyphenols-based diet in COVID-19 elderly and obese patients. Moreover, they examined the pathophysiology, clinical consequences, and disease indicators linked to senescence in COVID-19 patients. They showed that it is necessary to reach a better understanding of SARS-CoV-2 infection to treat or prevent severe COVID-19. Furthermore, they suggested that high levels of polyphenols could have a protective impact on COVID-19-related outcomes.

In a review by Giovinazzo et al. (52), the authors investigated the existing literature on the potential efficacy of polyphenols in the battle against SARS-CoV-2 infection. Even though this year has seen significant advancements in anti-inflammatory medicines, no effective cytokine blockers for COVID are presently being used in clinical settings. Accordingly, they showed that bioactive phytochemicals like polyphenols may prove to be effective adjuvants for reducing SARS-CoV-2 infection. Such nutrients, which share anti-inflammatory and antioxidant qualities with traditional anti-inflammatory medications, may aid in lowering inflammation in COVID-19 patients.

Majumder et al. (53) focused on the Mediterranean diet and on olive oil intake. Olive oil has anti-inflammatory and cardioprotective properties because it contains a variety of bioactive polyphenolic components, including oleanolic acid, oleuropein, oleocanthal, and HT. They also investigated the ongoing in silico research on the action of olive oil phytochemicals in inhibiting SARS-CoV-2 virus.

A review by da Silva et al. (54) aimed to summarize the most recent data from well-known potent flavonoid and non-flavonoid polyphenols derived from plant extracts—including catechin, quercetin, and kaempferol—that inhibit coronavirus strains in vitro or in silico. This research showed that the creation of novel coronavirus therapies, treatments/medicines, or formulations may benefit from the study of molecules outside of the established treatments.

A study by Paraiso et al. (55) reviewed the impact of polyphenols on COVID-19 therapeutic targets and offered insights into the potential use of polyphenols in the creation of all-natural treatments for this viral illness.

A mini-review by Abdelgawad et al. (12) showed that many in silico and in vitro studies have identified polyphenolic compounds found in olive tree leaves extract, such as oleuropein and HT, as anti-SARS-CoV-2 molecules. Additionally, they highlighted the anti-inflammatory, analgesic, immunomodulatory, and antithrombotic properties of olive leaf extract in a number of in vivo studies. These properties could be extremely helpful in the management of the COVID-19-associated inflammatory cytokine storm and disseminated intravascular coagulation.

Finally, a recent study by Annunziata et al. (56) studied the possibility that polyphenols may have additional anti-coronaviruses action to that already seen in vitro. They also shed some light on studies that have been published in the literature, which supported the idea that the primary general mechanisms of action behind the positive impact of polyphenols against coronaviruses are the decrease in viral titer and the suppression of nucleocapsid protein expression. Table 1 summarizes the main findings of the most relevant presented studies.

Conclusion

Olive tree polyphenols are bioactive molecules known for their antiviral activity against SARS-CoV-2 in silico, in vitro, and in vivo. These bioactive substances alter many signaling pathways and display a wide range of actions—including anti-inflammatory, antipyretic, immunomodulatory, and antithrombotic capabilities—in addition to their antiviral effects. Olive tree polyphenols offer a potentially safe natural source to treat the signs and symptoms of COVID-19 infection, including cytokine storms, and guard against negative outcomes. In this scoping review, we showed how polyphenols found in olive tree may help against COVID19 spread. Moreover, we shed some light on how the Mediterranean diet, which is notoriously rich in olives and olive oil, can help in the prevention of coronavirus infection. Finally, we presented how the work done in bioinformatics may reveal the mechanism of action of natural molecules, among which olive tree polyphenols.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Table 1. Review articles, in silico, in vitro, in vivo studies and clinical trials studying the effects and the mechanism of action of polyphenols from olive leaves for COVID-19 treatment.

Article Type	Reference	Aim of the Study
Review articles	(56)	They reviewed the antiviral action against coronaviruses of polyphenols from different sources, focusing on SARS-COV-2.
	(12)	They reviewed the beneficial activities demonstrated in in vivo studies of polyphenolic compounds found in olive tree leaves. These properties could be helpful in the management of COVID-19-associated cytokine storm and disseminated intravascular coagulation.
	(55)	They evaluated the impact of polyphenols on COVID-19 therapeutic targets and offered insights into the potential use of polyphenols in the creation of all-natural treatments.
	(54)	They reported the most recent data from well-known potent flavonoid and non-flavonoid polyphenols derived from plant extracts that inhibit coronavirus strains in vitro.
	(53)	They investigated the Mediterranean diet, the intake of olive oil, and the ongoing in silico research on olive oil's phytochemicals, which may be able to inhibit SARS-CoV-2 virus.
	(52)	They reviewed the existing literature on the potential efficacy of polyphenols against SARS-COV-2 infection.
In silico experimental articles	(36)	They found that secoiridoids may inhibit SARS-CoV-2.
	(37)	They looked for the inhibitory potential of betulinic acid and its activity against the main protease of SARS-CoV-2.
	(38)	They examined the role of thymopentin and oleuropein, which are phenolic compounds found in olive tree leaves, as potent inhibitors of SARS-CoV-2.
	(39)	They screened a library of 161 natural molecules from olive as inhibitors of tyrosine kinase, which is overexpressed in virus host cells.
In vitro experimental articles	(40)	They tested olive leaf hydroxytyrosol for its virucidal activity against SARS-CoV-2.
	(41–43)	They studied the effect of luteolin and kaempferol in preventing SARS-CoV-2 multiplication.
	(40)	They examined the anti-SARS-CoV-2 potential of hydroxytyrosol HT-rich aqueous olive pulp extract in vitro.
Epidemiological studies	(51)	They examined the anti-inflammatory effect of a polyphenol-based diet in COVID-19 elderly and obese patients. They also examined the pathophysiology, clinical consequences, and disease indicators linked to senescence in COVID-19 patients.
	(47)	They investigated the relationship between following the Mediterranean diet and COVID-19 cases and fatalities in a group of people from 17 areas of Spain.
	(49)	They presented the first epidemiological research that has linked a dietary pattern to COVID-19 severity in various subgroups patients.
Clinical trials	(50)	They examined the effects of plant-based and pescatarian diets against COVID-19 severity surveying small populations from six countries.
	(44)	They investigated the immunomodulatory and preventive effects of olive leaf tea against COVID-19 infection among 249 workers.
	(45)	They will conduct a clinical trial in which confirmed COVID-19 adult patients will be enrolled. Patients will be randomly assigned to receive, along with standard care, standardized olive leaves capsules or placebo, up to 10 days.
	(46)	They will investigate the effect of a dietary supplement containing olive polyphenols and flavonoids in preventing the progression of COVID-19.

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Review *Clin Ter 2023; 174 Suppl. 2 (6):149-153 doi: 10.7417/CT.2023.2481*

The Role of Olive Tree Polyphenols In The Prevention of COVID-19: A Scoping Review Part 2

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Abstract

The recent COVID-19 pandemic caused by SARS-CoV-2 affected hundreds of millions of people and caused millions of deaths. There are few effective medications against SARS-CoV-2, and several studies attempted to make drugs based on natural components, such as olive leaves. Olive leaves are rich in polyphenolic compounds, which were proposed as a viable co-therapy supplement to treat and improve clinical symptoms in COVID-19 patients. Polyphenols have renown anti-inflammatory and multitarget antiviral effects on several virus families, which could be among the reasons of the beneficial effects of the Mediterranean diet against COVID-19. This scoping review is focused on the effect of olive tree polyphenols as a natural remedy to inhibit SARS-CoV-2, mainly discussing their influence on the process of viral entry into host cells by endocytosis. ***Clin Ter 2023; 174 Suppl. 2 (6):149-153 doi: 10.7417/CT.2023.2481***

Key words: *SARS-CoV2, COVID-19 pandemic, polyphenols, antiviral, olive tree*

Introduction

In December 2019, a new coronavirus was identified in the city of Wuhan, China, in patients who had severe unexplained pneumonia (1). In February 2020, the World Health Organization (WHO) assigned the name of COVID-19 to designate the disease caused by this virus, initially called nCoV-2019 and then SARS-CoV-2 by the International Committee on Taxonomy of Viruses (2). After SARS-CoV-1 (China, 2002) and MERS-CoV (Arabian Peninsula, 2012), both responsible for fatal respiratory distress syndromes, this is the third global health threat linked to a coronavirus in less than twenty years (3).

SARS-CoV-2, like SARS-CoV-1, uses angiotensin-converting enzyme 2 (ACE2) as its main cell receptor in order to enter the host cell (4). Its incubation lasts about five days, leading in 70% of infected patients to respiratory symptoms like cough, fever, or dyspnea (5). After eight to ten days from the first symptoms, in some patients the viral infection is followed by an unsuitable immune reaction and inflammatory syndrome, marked by the worsening of respiratory symptoms (6). This dysimmune phase, called cytokine storm, can be associated with a coagulopathy, a life-threatening condition (7).

Currently, COVID-19 infection is treated with several medications, none of which can effectively cure the disease, but only alleviate the symptoms (8), (9). An effective drug should target the receptor ACE2, preventing SARS-CoV-2 from entering the cells, while also improving the host's immune system. Considering their widespread antiviral action, olive tree polyphenols have been proposed for SARS-CoV-2 treatment. Indeed, olive leaves extracts contain many polyphenolic components, such as hydroxytyrosol (HT), having antioxidant, anti-inflammatory, and antiviral properties (10)–(13).

The goal of this scoping review is to highlight the role of polyphenols in modulating inflammation and their antiviral effects against COVID19, which might help researches to find new insights for treatment to decrease viral infectivity and to fight the SARS-CoV-2 pandemic.

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Materials and Methods

Literature Scoping Review and Inclusion Criteria

The scoping review section followed PRISMA guidelines for scoping reviews (14). Our search included original and review articles published by MAGI laboratories focusing on SARS-CoV-2 and the effects of olive polyphenols and HT.

The articles employed had to be written in English and published in the period 2019-2022. Congress abstracts, manuscripts not written in English, and studies that were not relevant to the topic of the present manuscript were excluded.

Literature Search

PubMed database was searched to retrieve articles published from 2019 to 2022 that satisfied the inclusion criteria. The search keywords were: (MAGI(Affiliation) AND COVID-19 OR SARS-CoV-2 OR polyphenols OR hydroxytyrosol). In addition, the reference lists produced by the automated research were scanned manually to find further relevant scientific article.

Study Selection

All the resulting articles were assessed independently for eligibility by authors who evaluated titles and abstracts, according to the above inclusion criteria. Once a paper was found eligible, its references were screened to find new papers.

Results

Based on the literature review search criteria, 16 manuscripts published from 2019 to 2022 by the MAGI laboratories were found. Only 9 pertained to the topic of the current review and were thus read completely and discussed in the current manuscript. Their reference list was scanned for retrieving any other relevant scientific article.

The following sections will highlight the main findings of the included articles. Indeed, the antiviral and immunomodulatory activity of olive polyphenols, such as HT, and of α-cyclodextrin will be discussed. Moreover, the results of studies already performed, and the possible advantages of additional in vitro and in vivo studies or clinical trials studying the effects of natural molecules in fighting SARS-CoV-2 infection will also be discussed in this manuscript.

Polyphenols and Viral Infectivity

Viral infectivity depends on the interactions between components of the host cell plasma membrane and the virus envelope. Coronaviruses are a class of viruses with a long single positive RNA molecule and a lipid envelope, which requires a plasma membrane fusion process, mediated by endocytosis, a mechanism in which cholesterol and lipid rafts play a fundamental role. Identifying molecular targets that could block and inhibit the virus entry into the cell is important to decrease the viral activity of SARS-COV-2 (15, 16).

Baglivo et al. (17) presented a qualitative review on the importance of membrane molecules in coronavirus infectivity, and proposed their role as potential targets for lowering SARS-COV-2 infectivity. The authors of the study have focused on the involvement of lipid structures—such as cholesterol and lipid rafts—in the endocytosis-mediated process of viral attachment and cell infection. Moreover, they discussed a variety of naturally occurring compounds, including cyclodextrin and sterols, that can decrease the infectiousness of a variety of viruses, including members of the coronavirus family, by interfering with their lipid-dependent attachment to human host cells.

Natural compounds that may inhibit viral entry into host cells by endocytosis were subsequently reviewed by Kiani et al. (18). The authors showed that in recent years natural remedies for viral infections have become more significant, and that many natural substances, such as phytosterols, polyphenols, flavonoids, citrus, galangal, curcuma, and hydroxytyrosol (HT), are being studied to determine whether they may suppress SARS-CoV-2.

The Antiviral Activity of Hydroxytyrosol and α-Cyclodextrin and the Molecular Docking Studies to Evaluate Their Interaction with SARS-Cov-2

HT is a natural molecule, having anti-inflammatory, antitumor, antiviral, antibacterial, and antifungal properties, that can be extracted from olive leaves and fruits (19). HT also reduces the serum lipids in mice fed with high-cholesterol diets, indirectly modifying the composition of their plasma membrane (18). Furthermore, HT improves endothelial dysfunction, decreases oxidative stress, and is neuro- and cardio-protective. Due to all these biological properties, HT is currently one of the most actively investigated natural phenols, with a great pharmacological potential (19).

α-cyclodextrin is a natural molecule, produced by bacteria that can deplete sphingolipids and phospholipids from cell membranes. Indeed, cyclodextrins have been exploited by many researchers to replace membrane leaflets with exogenous lipids. Moreover, α-cyclodextrin can reduce serum concentration of phospholipids, reducing viral endocytosis processes (20).

Considering all the properties of HT and α-cyclodextrin, it has been decided to study them in silico, in order to determine their interactions with lipid-raft-mediated endocytosis of SARS-CoV-2. In two articles by Paolacci et al. (20) and Ergoren et al. (21), the authors firstly reviewed the role and interactions of HT and α-cyclodextrin in lipid-raft-mediated endocytosis of SARS-CoV-2. Then, thanks to in silico studies, they demonstrated that α-cyclodextrin and HT interact with the viral spike protein and its host cell receptor ACE2, suggesting an impact on the SARS-CoV-2 endocytosis process.

HT and α-cyclodextrin were finally tested in vitro and in vivo for their anti-SARS-CoV-2 properties. In the study by Paolacci et al (22), in vitro and clinical studies on the efficacy of α-cyclodextrin and HT against SARS-CoV-2 infection were examined. Both the in vitro analysis (performed on Vero E6 cells, Caco2, and human fibroblast cell lines) and

the clinical studies, which recruited 149 volunteers and 76 controls, proved that HT and α-cyclodextrin increase defenses against SARS-CoV-2 infection and reduce the synthesis of viral particles.

Apart from SARS-CoV-2, HT and α-cyclodextrin were tested for preventing the proliferation of oral pathogens due to prolonged face mask use. HT and α-cyclodextrin proved to reduce the growth of bacteria and fungi and also halitosis and gingival and mouth inflammation in several volunteers. Thus, it can be deduced that it is safe and beneficial to use α-cyclodextrin and HT to lessen the bacterial and fungal load brought on by frequent use of face masks (23).

HT α-Cyclodextrin as Oral Spray to Fight SARS-CoV-2 Infection

A study by Paolacci et al (24) presented an observational study to evaluate the safety profile of the "Endovir Stop" spray. The authors proposed a mouth spray that might stop SARS-CoV-2 endocytosis, based on HT and α-cyclodextrin. The spray was tested on 87 healthy subjects; also, the cytotoxicity and antioxidant capacity of the spray was evaluated in vitro. From the results, the spray is not cytotoxic and it has a good antioxidant capability. Moreover, the clinical tests on healthy volunteers revealed that there were no adverse effects and no medication interactions while receiving therapy, proving the safety of "Endovir Stop" spray.

The effectiveness of an oral spray containing α-cyclodextrin and HT against SARS-CoV-2 transmission was tested in a pilot study carried out by Ergoren et al (21). They recruited 50 healthy volunteers at a higher risk of SARS-CoV-2 infection from Northern Cyprus and 6 individuals that tested positive for SARS-CoV-2. Despite being at a greater risk of infection than the general population, the 50 healthy volunteers did not test positive for SARS-CoV-2 after receiving the spray for two weeks. Interestingly, despite the viral load being larger in the treated individuals than in the untreated patients who became negative after ten days, 2 of the cohort's 6 positive patients went from positive to negative within five days. Moreover, they made an in silico prediction to evaluate the interactions of HT and α-cyclodextrin with proteins involved in SARS-CoV-2 endocytosis. They discovered potential interactions between HT and α-cyclodextrin and the human cell proteins Spike, ACE2, and TMPRSS2. To conclude their pilot study, they mentioned that their findings suggested a potential contribution of HT and α-cyclodextrin in strengthening immune defenses against SARS-CoV-2.

The Role of Polyphenols in Treating Post-COVID Syndrome

A proportion of COVID-19 patients experience post-COVID fatigue, persistent enervating symptoms and post-exertional neuroimmune exhaustion similar to those observed in SARS patients. This condition was consequently named "post-COVID syndrome" (PCS) (25). PCS is characterized by persistent multi-organ damage due to severe inflammatory responses, oxygen deprivation, thrombotic microangiopathy, and venous thromboembolism (26). However, there are currently few reports on the mechanisms underlying PCS, and it remains extremely difficult to understand why some people recover quickly while others develop the syndrome. In certain cases, prolonged illness seems to be linked to older age and multiple chronic medical conditions (27).

In a study by Naureen et al. (28), the authors reviewed data on post-COVID syndrome, in order to emphasize its etiological cause and the dietary regimens and supplements that might lessen or remove the associated chronic fatigue, gastrointestinal problems, and ongoing inflammatory responses. The authors selected acetyl L-carnitine, HT, and vitamins B, C, and D as possible natural molecules that show great potential as dietary supplements for the treatment of post-COVID syndrome. Thus, they begun a pilot observational trial, evaluating how HT, acetyl L-carnitine, and vitamins B, C, and D affected individuals who, despite recovering from COVID-19, were still experiencing post-COVID syndrome, obtaining encouraging findings.

Conclusion

Few medications and vaccinations are currently on the market for COVID-19 treatment, and scientific research is now working on finding new effective molecules. The main goal of this scoping review is to present studies involved in finding potential molecular targets and natural molecules to stop the spread and interrupt SARS-CoV-2 transmission. Early on, during a coronavirus infection, endocytosis occurs, and it is directly connected to viral contagiousness. The effects of inhibitors, such as cyclodextrin and phytosterols, as well as naturally occurring inhibitors like flavonoids, α- cyclodextrin and HT, are examined. Various molecular targets implicated in this process, including ACE2 receptors, lipid rafts, and proteases, are investigated. In particular, HT appear to hold great potential for the development of COVID-19 treatment approaches.

Acknowledgements

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Conflicts of interest statement

Authors declare no conflict of interest.

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Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) Checklist

Title	**1**	**Identify the report as a scoping review.**	**1**
ABSTRACT			
Structured summary	2	Provide a structured summary that includes (as applicable): background, objectives, eligibility criteria, sources of evidence, charting methods, results, and conclusions that relate to the review questions and objectives.	1
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known. Explain why the review questions/objectives lend themselves to a scoping review approach.	2
Objectives	4	Provide an explicit statement of the questions and objectives being addressed with reference to their key elements (e.g., population or participants, concepts, and context) or other relevant key elements used to conceptualize the review questions and/or objectives.	2
METHODS			
Protocol and registration	5	Indicate whether a review protocol exists; state if and where it can be accessed (e.g., a Web address); and if available, provide registration information, including the registration number.	N.A.
Eligibility criteria	6	Specify characteristics of the sources of evidence used as eligibility criteria (e.g., years considered, language, and publication status), and provide a rationale.	2-3
Information sources*	7	Describe all information sources in the search (e.g., databases with dates of coverage and contact with authors to identify additional sources), as well as the date the most recent search was executed.	2-3
Search	8	Present the full electronic search strategy for at least 1 database, including any limits used, such that it could be repeated.	2-3
Selection of sources of evidence†	9	State the process for selecting sources of evidence (i.e., screening and eligibility) included in the scoping review.	2-3
Data charting process‡	10	Describe the methods of charting data from the included sources of evidence (e.g., calibrated forms or forms that have been tested by the team before their use, and whether data charting was done independently or in duplicate) and any processes for obtaining and confirming data from investigators.	N.A.
Data items	11	List and define all variables for which data were sought and any assumptions and simplifications made.	2-3
Critical appraisal of individual sources of evidence§	12	If done, provide a rationale for conducting a critical appraisal of included sources of evidence; describe the methods used and how this information was used in any data synthesis (if appropriate).	N.A.
Synthesis of results	13	Describe the methods of handling and summarizing the data that were charted.	2-3
RESULTS			
Selection of sources of evidence	14	Give numbers of sources of evidence screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally using a flow diagram.	3-5
Characteristics of sources of evidence	15	For each source of evidence, present characteristics for which data were charted and provide the citations.	3-5
Critical appraisal within sources of evidence	16	If done, present data on critical appraisal of included sources of evidence (see item 12).	N.A.
Results of individual sources of evidence	17	For each included source of evidence, present the relevant data that were charted that relate to the review questions and objectives.	3-5
Synthesis of results	18	Summarize and/or present the charting results as they relate to the review questions and objectives.	3-5
DISCUSSION			
Summary of evidence	19	Summarize the main results (including an overview of concepts, themes, and types of evidence available), link to the review questions and objectives, and consider the relevance to key groups.	3-5
Limitations	20	Discuss the limitations of the scoping review process.	5
Conclusions	21	Provide a general interpretation of the results with respect to the review questions and objectives, as well as potential implications and/or next steps.	5
FUNDING			
Funding	22	Describe sources of funding for the included sources of evidence, as well as sources of funding for the scoping review. Describe the role of the funders of the scoping review.	5

JBI = Joanna Briggs Institute; PRISMA-ScR = Preferred Reporting Items for Systematic reviews and Meta-Analyses extension for Scoping Reviews.
* Where sources of evidence (see second footnote) are compiled from, such as bibliographic databases, social media platforms, and Web sites.
† A more inclusive/heterogeneous term used to account for the different types of evidence or data sources (e.g., quantitative and/or qualitative research, expert opinion, and policy documents) that may be eligible in a scoping review as opposed to only studies. This is not to be confused with information sources (see first footnote).
‡ The frameworks by Arksey and O'Malley (6) and Levac and colleagues (7) and the JBI guidance (4, 5) refer to the process of data extraction in a scoping review as data charting.
§ The process of systematically examining research evidence to assess its validity, results, and relevance before using it to inform a decision. This term is used for items 12 and 19 instead of "risk of bias" (which is more applicable to systematic reviews of interventions) to include and acknowledge the various sources of evidence that may be used in a scoping review (e.g., quantitative and/or qualitative research, expert opinion, and policy document).

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Review

Clin Ter 2023; 174 Suppl. 2 (6):154-158 doi: 10.7417/CT.2023.2482

Olive tree polyphenols as effective and sustainable grain preservatives

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Abstract

Whole grains play a crucial role in the human diet. Despite being cultivated in distinct regions, they are shipped everywhere, therefore making biosafety and security essential throughout the grain industry, from harvest to distribution. Phytopathogens, which have an impact on crop yield, induce grain spoiling and reduce grain quality in a number of ways, providing a constant danger to crop storage and distribution. Chemical control approaches, such as the use of pesticides and fungicides, are detrimental to the environment and hazardous to human health. The development of alternative, environmentally friendly, and generally acceptable solutions to ensure increased grain yield, biosafety, and quality during storage is crucial in order to guarantee sufficient food and feed supplies. As a means of self-defense against microbial infection and spoilage, plant matrices feature antimicrobial natural chemicals, which have led to their widespread usage as food preservatives in recent decades. Olive tree extracts, known for their high polyphenol content, have been widely used in the food preservation industry with great success, and are highly welcomed by people all over the world. In addition to their well-known health advantages, polyphenols are a valuable plant secondary metabolite because of their great antibacterial capabilities as natural preservatives. This article discusses the promising usage of polyphenols from olive trees as a natural alternative preservative, while also highlighting the future of olive eaves in the food industry. ***Clin Ter 2023; 174 Suppl. 2 (6):154-158 doi: 10.7417/CT.2023.2482***

Key words: *polyphenols, grain preservative, olive tree, natural molecules, antimicrobial*

Introduction

Humans heavily rely on cereal grains (like wheat, corn, rye, oat, rice, etc.) for sustenance and energy. However, cereals are subjected to a wide variety of biotic and abiotic stress factors during their development and storage. In particular, the cereal industry faces significant challenges from pathogenic and spoilage microorganisms such as bacteria, yeasts, and filamentous fungus (1). The quality of grains must be maintained during storage, transit, and conveyance, with the two extremes being short-term storage on the farm for drying and long-term storage for strategic reserves. To clarify, either on-farm or large-scale commercial storage options are viable (2). As the world's population rises, so does the demand for cereals; and also the amount of grain lost in storage rises to roughly 20% of the total production (3, 4).

Foods containing grains that have been contaminated with microbes pose a risk to human and animal health (4), so, the direct and indirect effects of fungal infection in stored food grains on food economies are of worldwide concern (2), with diseases wiping off about 20% of a year's worth of wheat production (3, 5). Mycotoxins, which are released by a wide variety of seed-borne fungus and can lead to a wide range of health issues in consumers, are another worry (5): for example, ochratoxin A, a mycotoxin of Aspergillus ochraceus, is found in 25-40% of cereals consumed worldwide (6, 7). Furthermore, the European Food Safety Authority's Panel on Contaminations in the Food Chain reported that grains and grain-based products are one of the three main chronic dietary sources of ochratoxin A. Aspergillus flavus is the primary source of the most hazardous mycotoxins, aflatoxins, and an estimated 4.5 billion individuals in developing countries are at risk of contracting aflatoxicoses (7).

However, the duration of storage, the water content of grains, the storage temperature, the humidity during storage, and the type of storage technology are all significant contributors to the development of mold and other types of fungi (8, 9). However, Aspergilli may have a greater prevalence than other fungi because of their saprophytic nature and capacity to colonize numerous substrates due to their secretions of various hydrolytic enzymes (11). This may explain why Fusarium spp., Aspergillus spp., and Penicillium spp. were shown to be dominant. The synthesis of fumonisins (Fusarium mycotoxins) has been observed to occur post-harvest, when storage conditions are poor (14), despite the fact that Fusarium species are typically thought

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of as field fungus. One of the greatest issues faced by food scientists is getting mycotoxins out of the food supply. Therefore, it is important to take measures to eliminate mycotoxin sources, such as mycotoxin-producing fungi, from the stored grains (8).

In order to achieve this goal, farmer employ many kinds of pesticides in large quantities. However, the increased use of pesticides has led to many side effects, such as pest resistance, outbreaks of novel pests, toxicity to non-target species, and adverse impacts on the environment (11). Synthetic pesticides play a crucial role in crop protection nowadays. Grain disinfection during storage is often accomplished using phosphine (PH3) or methyl bromide (CH3Br); however, the use of the latter is banned in Europe and in the United States (13). Therefore, novel fungicides/preservatives that both have enhanced performances and are also environmentally benign are necessary (12). Hermetic storage, microwave heating, gaseous ozone applications, cold plasma, ionizing radiation, pulsed light, or supercritical carbon dioxide (SCeCO2) are just a few of the novel control measures that have been extensively studied as nonchemical fungi management practices (8).

Plants are thought to have the greatest concentration of bioactive secondary metabolites that might be used as natural food preservatives: flavonoids and non-flavonoids, terpenes, aldehydes, ketones, aliphatic alcohols, organic acids, thiosulfinates, saponins, and glucosinolates are some of the most common types of active chemicals found in plants, herbs, and spices. New research shows that the bioactive chemicals found in plant extracts and essential oils can impede the growth or activity of foodborne pathogens (bacteria, yeasts, moulds, viruses). Using plant extracts and essential oils to combat seed-associated fungi might be a sustainable alternative that can also reduce the likelihood of disease resistance (10, 12). The antibacterial activity of many plant extracts has been the subject of several researches, showing their potential as synthetic preservative alternatives. As a result of this rising demand, several organizations and governments are pouring resources into research and development of all-natural food preservatives (15).

Methodology

This paper is a "narrative review". All the information used for its preparation is based on data from original documents and reviews. We selected the most relevant studies on olive tree polyphenols and their roles as antimicrobial substances and preservatives. We conducted an electronic search in MEDLINE, PubMed database, Google Scholar and Scopus, using the following string: (olive polyphenols(Text Word) OR polyphenols(Text Word)) AND (antimicrobial(Text Word) OR preservatives(Text Word)). The reference lists of selected articles were scanned to retrieve additional relevant research.

Polyphenolic profile of Olive Tree

In plants, the shikimate and polyketide pathways are responsible for the production of polyphenols, which are non-nutritional secondary metabolites and one of the most pervasive classes of plant-based chemicals.

There are both simple and complex phenolic compounds in olive fruit: these phenolics are responsible for the oxidation resistance and organoleptic qualities of the oil (16). Olive oil contains both simple and complex phenols, in concentrations of up to 1% by weight. Five to ten percent of the total contents of the olives is released into the oil during production (crushing), while the vast majority stays in the water phase (vegetation water) like hydroxytyrosol, tyrosol, and the lipid soluble oleuropein and ligstroside aglycones. About 90% of olive phenols are transported to the water phase during the pressing of the drupes, making vegetable water a significant source of phenolic antioxidants (1-1.8% w/v) (16).

It has been established that hydroxytyrosol, also known as 3, 4- dihydroxytyrosol or 3, 4-dihydroxyphenylethanol, is the primary phenolic component of olive extract and olive oil, and its presence has been detected and measured also in wines (17). Both hydroxytyrosol and oleuropein aglycone (an ester of hydroxytyrosol and elenolic acid) may be found in olive oil. Hydroxytyrosol in its purest form is a colourless, odourless, and flavourless liquid that can be either hydro- or lipo-soluble. It has the highest ORAC value of any phenolic antioxidant found in olive oil, making it the most powerful olive oil antioxidant. Considering several beneficial effects of hydroxytyrosol, it has been proposed as a dietary supplement for many conditions (18-21).

Moreover, the olive tree and other members of the Oleaceae family contain a phenolic secoiridoid glycoside, called oleuropein. This compound may be found in the tree's bark, leaves, and fruit. A bitter glycoside accounts for up to 14% of the dry weight of the drupe, making it the most prevalent phenolic compound in the fruit(17).

Effect of Oleuropein on Microorganisms

The antibacterial properties of oleuropein extend to a wide variety of microorganisms, including viruses, retroviruses, bacteria, yeasts, fungi, molds, and other parasites (22-25). Research has established that oleuropein has an antibacterial action against *Salmonella enteriditis*, and that it inhibits sporulation in *Bacillus cereus* (26,27). In addition, olive leaves were shown to have an antibacterial action against *Escherichia coli* and *Candida albicans*, and were particularly effective against Klebsiella and Pseudomonas, two bacterial taxa that represent severe resistance problem (25,28). A recent U.S. patent claims that oleuropein effectively inhibits the replication of herpes mononucleosis virus, hepatitis virus, rotavirus, bovine rhinovirus, canine parvovirus, and feline leukaemia virus (29).

The antimicrobial activity of oleuropein and hydroxytyrosol against American Type Culture Collection and clinical bacterial strains (*Haemophilus influenzae*, *Moraxella catarrhalis*, *Salmonella typhi*, *Vibrio parahaemolyticus*, and *Staphylococcus aureus*) was demonstrated by Bisignano et al. (30). Possible anti-HIV properties of the olive leaf have also been studied. These results demonstrated for the first time that oleuropein and hydroxytyrosol are part of a class of small molecules that can take on multiple roles in fighting the

AIDS virus, blocking the virus's ability to enter and integrate into cells both in and out of the body (31-33).

Preservative Properties of Olive Polyphenols

Recently, the consumers' preference for foods that have had minimum processing and appear to be natural and healthy have prompted an active quest for plant-derived natural preservation agents (33). These should be used instead of conventional preservatives, to increase food safety while also extending its shelf life (34). Due to their low toxicity and costs and thanks to their widespread availability, phenolic extracts of olive leaves and fruits have been the matter of various researches on food preservation. Due to olive oil's high antioxidant content and other health benefits, its production has increased in response to the rising demand from the Mediterranean diet. Therefore, a serious environmental hazard is posed by the massive quantities of waste created during the manufacture of Extra Virgin Olive Oil, specifically olive oil mill wastewaters. Olive oil mill wastewaters is appealing as a powerful source of natural antioxidants due to its high concentrations of sugars, nitrogenous chemicals, volatile acids, polyalcohols, pectins, lipids, and polyphenols. Therefore, research is being conducted to find the best ways to extract polyphenols from olive oil mill wastewaters and put them to good use (35, 36).

Olive trees have evolved a defensive system that involves the production of secondary metabolites, like polyphenols, in response to attacks by microorganisms and other animals. A number of studies has shown that polyphenols can be effective in preventing the growth of bacteria, yeast, and fungi that cause food deterioration (37–39). Plant polyphenols have also been shown to have antibacterial action, which supports their potential application as food preservatives. Today, polyphenols may be recovered from olive mill effluent by industrial valorization (40). For instance, at least five firms throughout the globe extract polyphenols from olive mill effluent and market them as bioactive additives or natural preservatives to be used in food and cosmetics. Several uses have been proposed for polyphenols, due to their potential as a food preservative. Bacteria are less likely to acquire resistance to the polyphenols when they are administered in conjunction with other antimicrobials and antibiotics, because each bioactive polyphenol has a unique action mechanism. Thus, polyphenols have the potential to halt this rising tide of drug-resistant bacteria and to protect consumers from their potentially harmful effects (41).

Polyphenol Safety Evaluation and Future Perspectives

There is a widespread misconception that any amount of naturally occurring chemical, no matter how much of it is consumed, poses no danger to one's health. As a result, currently only a limited amount of information is accessible in the scientific literature about the possible risks associated with the consumption of plant extracts. In addition, the long-term safety of taking significant doses of polyphenols as dietary supplements or food additives is not well understood in humans, and more studies need to be done on the topic before it can be considered established. The beneficial effects of polyphenols have been demonstrated by a number of experiments carried out on animals, despite the fact that the conclusions drawn from several sub-chronic and oral toxicity tests are still up for discussion. Because of the possibility of cytotoxicity posed by polyphenols and other naturally occurring compounds, it is absolutely necessary to determine what quantities are appropriate to use as food preservatives. The oral administration of proanthocyanin-rich grape seed extracts to rats at doses as high as 2 and 4 g/kg was shown to be safe in the study's evaluation of both acute and subchronic toxicity. The rats were administered the extracts via the gastrointestinal tract. On day 14 of the clinical study, during the acute examination, it was discovered that the lethal dosage was over 4 g/kg (42).

Extensive research on humans is required in order to ascertain the optimal dosage for risk-free use and to reduce the likelihood of potentially harmful adverse effects. A better knowledge of evolving safety issues will also be substantially supported by recent breakthroughs in omics techniques and strategies, as well as more methodically prepared animal trials. This is because both of these areas of research have been expanding rapidly in recent years. Deeper research into the structure-activity relationship of natural antimicrobials and their modes of action may help pave the way for their application in a wider range of food systems. Natural antimicrobials have the potential to prevent antibiotic resistance. Last but not least, regulatory organizations have to analyze food applications and guarantee safe long-term levels in order to safeguard consumers.

Conclusion

There is a lot of evidence suggesting that polyphenols, which are natural secondary metabolites, are good for human health; as a direct consequence of this, an increasing number of individuals are including polyphenol supplements into their dietary regimens. In addition, polyphenols have gained a substantial amount of interest as natural antimicrobial preservatives, representing a viable alternative to synthetic pesticides for inactivating crop spoilage and pathogenic bacteria spreding, while also increasing microbiological safety. This is mostly due to the rising desire among consumers for minimally processed and healthier foods. Since olive phenols provide several benefits for stored grains, including color preservation, growth of microbiological organisms reduction, and enhanced storage stability, there is a great amount of potential for their use as preservatives in the cereal grain business. Extensive research is also required to establish the safest dose that may be used in a range of healthy meals without creating unintended side effects.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review *Clin Ter 2023; 174 Suppl. 2 (6):159-168 doi: 10.7417/CT.2023.2483*

Unraveling the Role of Prickly Pear Extract as a Potent Nutraceutical Agent Against Metabolic Syndromes

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Abstract

Background. Prickly pear (Opuntia) extracts have garnered considerable attention in recent years due to their promising medicinal and nutritional properties. This comprehensive review explores the multifaceted potential of prickly pear extracts in mitigating various chronic diseases, including cardiovascular diseases (CVDs), diabetes, obesity, cancer, neuronal diseases, and renal diseases.

Methods. This review provides a comprehensive overview of the diverse therapeutic applications of Opuntia extracts in managing chronic diseases. The collective evidence underscores the potential of prickly pear as a valuable natural resource for addressing global health challenges. Further research and clinical investigations are warranted to unlock the full potential of Opuntia in the prevention and treatment of chronic diseases.

Results. Studies have suggested that the bioactive compounds within prickly pear may influence glucose metabolism by improving insulin sensitivity, reducing insulin resistance, and modulating gut microbiota composition. These pathways exhibit potential in the reduction of hyperglycemia, which is a fundamental aspect of metabolic syndromes. Opuntia extracts demonstrate also antioxidant, anti-inflammatory capabilities that can contribute to improving health in various conditions.

Conclusion. Further research and clinical investigations are warranted to unlock the full potential of Opuntia in the prevention and treatment of chronic diseases. ***Clin Ter 2023; 174 Suppl. 2 (6):159-168 doi: 10.7417/CT.2023.2483***

Key words: *Opuntia, Bioactive Compounds, Prickly Pear, Nutraceutical, Metabolic Syndrome, Traditional Medicine, Nutrition*

Introduction

The phrase "metabolic syndrome" encompasses a cluster of medical conditions, namely atherogenic dyslipidemia, systemic hypertension, central obesity, reduced levels of high-density lipoprotein cholesterol, hypertriglyceridemia, and insulin resistance (1, 2). Adults make up 20-25% of the population with metabolic syndrome (3, 4), whereas children make up 0-19.2% (5). In addition, the prevalence of such disorders is associated with a higher risk of developing several diseases (6). The present investigation focuses on the examination of natural substances as an alternative to synthetic molecules, with the aim of reducing expenses and potential adverse consequences associated with the latter. It is therefore recommended to use nutraceuticals, which are bioactive chemicals derived from plants and have significant nutritional benefits. An intriguing source of plant bioactive substances is the *Opuntia* plant, a kind of cactus that is typical of the Mediterranean region (7).

There are about 1,500 different species of cacti, all of which are members of the Cactaceae family (dicotyledonous angiosperms) (8). In addition to the medicinal chemicals it contains, *Opuntia* is also a useful plant in the kitchen, being used for making jams, juices, alcoholic drinks, and natural liquid sweeteners (9). They are also utilized in wastewater treatment (10), agrochemicals, cosmetics, and in the chemical industry (11). In traditional medicine, prickly pear is sometimes referred to as *Opuntia ficus-indica* (OFI), fig *Opuntia*, or "the Indian fig".

Opuntia has been domesticated in numerous places, including India, Africa, Australia, the Middle East, Latin America (12), and the Mediterranean areas (13). It is a perennial shrub that grows slowly and can reach a height of 3-5 m (14). Nutraceutical and health-enhancing applications involve the use of OFI plant components, mainly due to the fact that they have many beneficial effects on glucose homeostasis, oxidative stress, and metabolism (15, 16). According to a meta-analysis, prickly pear consumption is associated with significant reductions in body fat, blood pressure, and cholesterol (17). The most notable effects of OFI are its effectiveness in treating a variety of viral infections, allergies, diabetes, hypertension, cardiovascular disorders, prostatitis, wounds, tumors, warts, etc. (18). Considering the aforementioned information, this article evaluates the possible biological effects and health advantages of prickly pear as a nutraceutical in a number of metabolic diseases.

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Nutritional Composition of Prickly Pear Extract

The fruit of the prickly pear is a popular crop. It has an appealing taste and contains a significant quantity of vitamins, minerals, carotenoids, flavonoids, phenolic acids, biopeptides, fiber, betalin, and phytochemicals (19).

Over the past 10 years, the prickly pear fruit has drawn some interest particularly due to its high concentration of betalain and phenolic components, which are primarily responsible for its health-promoting characteristics due to their antioxidant and anti-inflammatory potential (20). Also, extracts from fruits with red skin contain betalins, ascorbic acid, carotenoids, flavonoids (such as luteolin, kaempferol, isorhamnetin, and quercetin), and taurine, but also compounds that can scavenge free radicals. Consequently, red fruits are beneficial to human and animal health because of their antioxidant components (21). According to studies, scavenging action increases with the content of polyphenols (22). Cactus flower extract contains flavanol glycosides, which have antioxidant characteristics and find applications as a food, drug, and cosmetic component (23).

The pulp, seeds, and skin of prickly pears have been shown to contain compounds such as limonene, esadecanic acid, squalene, and carvacrol, which have antioxidant and antimicrobial properties that prevent the entry of pathogenic agents in the body (24). According to extensive research, prickly pear contains significant quantities of calcium, magnesium, potassium, and phosphorus, as well as a diverse array of micro- and macro-minerals, making it an abundant source of these essential nutrients (25). Additionally, prickly pear peel also contains sterols, neutral glycolipids and phospholipids (26), 17-Decarboxy betanin and betanin (27), xanthophyll, and chlorophyll (28, 29).

Mechanisms of Action

Many studies have been undertaken throughout the years to investigate the inflammatory process-related potential targets of OFI (30-35). The majority of research papers examining the anti-inflammatory effects of *Opuntia sp*. have utilized crude extracts, with just a limited subset of these studies employing this approach (30, 36, 37) offering information on the capacity to reduce inflammation of isolated phenolic compounds, especially flavonoids from *Opuntia sp*. derivatives (isorhamnetin and kaempferol). Polyunsaturated fatty acids found in the oil extracted from prickly pear seeds (38) and phenolic compounds (9), essential oils (10), and pigments (8) from its fruit are the main constituents of this plant that show anti-inflammatory activity.

Mammalian tissues experience inflammation as a normal self-regulatory reaction to adverse conditions, such as microbial invasion, physical injury, or exposure to toxic substances. By facilitating a well-organized immune response—notably, macrophages and mast cells—the inflammatory process strives to eradicate the main trigger and helps to start the regeneration of wounded tissues (39, 40). However, there are instances in which the mechanisms responsible for the restoration of tissue homeostasis fail to function properly, leading to a deregulated response that frequently results in a chronic inflammatory response (39, 41). During the inflammatory process, biological systems (cells like macrophages and mast cells, and signaling molecules) and pro- and anti-inflammatory mediators all work together in a complicated cascade (42, 43). Most research investigating the anti-inflammatory potential of *Opuntia sp.* extracts and its purified compounds was conducted in vitro, focusing on mediators and enzymes that regulate inflammatory reactions upon any encounter with inflammatory stimuli—i.e., phenolic compounds including flavonoids and phenolic acids (44).

Most of the studies based on determining the anti-inflammatory potential of *Opuntia sp.* compounds and extracts reported that certain enzyme mediators take part in the process of inflammation: for instance, nitric oxide (NO), which is created by the inducible nitric oxide synthase (iNOS) (44). The pulp and peel of two types of prickly pears showed anti-inflammatory potential by scavenging NO radicals, according to Gómez-Maqueo and colleagues (45). In fact, elevated NO levels are involved in a variety of physiological activities, as well as in the pathophysiology of inflammation, by promoting the synthesis of pro-inflammatory cytokines (TNF-αand IL-8) and iNOS, ultimately resulting in substantial tissue damage (34, 46). Further investigation by the same researchers revealed that several prickly pear components may impede hyaluronidase function. By hydrolyzing hyaluronic acid (HA), one of the most significant components of the extracellular matrix (ECM), this enzyme is associated with both pathogenic and physiological activities (47). HA breakdown by hyaluronidase causes tissues to lose their firmness and thus makes them more permeable, which encourages the release of inflammatory mediators. Therefore, it is essential to keep hyaluronic acid metabolism under control, in order to sustain the ECM structure and ensure proper tissue organization. The inhibitors of this enzyme, such as prickly pear, might prove useful amid the inflammatory process, offering possible health advantages like reducing the worsening of the inflammatory response (47-49). Phenolic compounds also showed their anti-inflammatory potential by scavenging free radicals. Similar bioactivities were examined in vitro in different investigations, using various prickly pear cultivars, and peels showed notably more efficacy than pulps. This behavior was specifically attributed to phenolic substances such as glycosides, isorhamnetin, kaempferol, quercetin, and indicaxanthin (44). These compounds were found to exhibit anti-inflammatory activity, by showing a stronger hyaluronidase inhibitory activity than other pure standards (50).

A study conducted by Chaalal et al. showed that following lipopolysaccharide (LPS) stimulation, polyphenols obtained from various prickly pear fruit components show anti-inflammatory action, and may have a neuroprotective effect by lowering the expression of pro-inflammatory mediators like TNF-α, IL-1β, and iNOS in N13-microglial cells at the transcriptional level (51). Filannino and colleagues reported that fermented extracts and raw material from OFI cladodes demonstrated an anti-inflammatory effect by substantially lowering the production of significant effectors (NO and chemokine TNF-α and IL-8) of the inflammation process, contributing to the recruitment as well as activation of various cells associated with inflammation. Flavonoids—particularly isorhamnetin and kaempferol,

which also reduced the intracellular ROS produced during cells' activation—were found to be the primary contributors to this anti-inflammatory modulation. They showed a considerable reduction in the accumulation of prostaglandin E2, a pro-inflammatory product generated in the COX-2 and prostaglandin synthase metabolic pathway (52).

Similar to this, Matias et al. conducted an experiment and found that an *OFI* fruit, rich in flavonoids, not only reduced oxidative stress by scavenging free radicals produced because of H2O2, but also prevented the oxidation of protein in inflamed Caco-2 cells. They also found that the extract significantly affected the release of cytokines from inflamed Caco-2 cells; in particular, it reduced the synthesis of IL-8 and NO, which are linked to activating the NF-B pathway (one of the major inflammatory signaling pathways). On the other hand, the extract also prevented intestinal barrier malfunction, which was linked to some flavonoids' capacity to reduce TNF-α secretion. They discovered that the extract greatly altered the release of cytokines from inflamed Caco-2 cells, specifically causing a reduction in the production of IL-8 and NO, which are connected to the NF-B pathway activation. It was discovered that the extract prevents the ubiquitination of IκBα, a crucial NF-κB inhibitor, thereby preventing it from migrating from cytosol to the nucleus, where it may induce the transcription of pro-inflammatory genes (53).

The majority of research conducted on extracts and isolated components of *Opuntia sp*. has been carried out in controlled laboratory environments. The main focus of these studies has been examining particular mediators and enzymes that play a role in the inflammatory process, as outlined in Table 1.

The Role of the Biological and Medical Properties of Prickly Pear in Mitigating Chronic Diseases

Researchers have extensively investigated *Opuntia* extracts for their medicinal and nutritional benefits, examining their medicinal potential in both in vitro and in vivo studies (56). Prickly pear extracts have potential therapeutic applications in various diseases, including diabetes, cardiovascular diseases, neuronal diseases, obesity, cancer, and others.

Cardiovascular Diseases

The most common group of death-causing diseases worldwide are cardiovascular diseases (CVDs) (57). The death rate due to CVDs has been decreasing in Western countries since the start of the last decade, but its death rate is increasing in developing countries. In the last 30 years, deaths due to CVD have increased to 11% in Mexico (58): in this country, the risk factors for CVDs—such as obesity, hypertension, diabetes, increased levels of cholesterol, and smoking—match those that have also been reported in Western countries (59, 60). CVDs have been also correlated to many genetic disorders (61-67). *Opuntia* has many nutritional benefits for CVD treatment, as it also prevents their formation and development (68). This important role of prickly pear can be explained by its antiatherogenic property, thanks to which the atherosclerosis risk can be reduced by the high amount of antioxidants this plant contains. These antioxidants can be obtained from the juice and fruit of the prickly pear, and result in reducing oxidative stress (69, 70). The increased content of polyphenols (antioxidants) in this plant also helps in reducing the peroxidation of lipids, which will decrease the formation of atherosclerosis.

Table 1. Opuntia indica's phenolic compounds are studied for their anti-inflammatory effects and potential mechanisms of action.

Models	**Phenolic Compounds**	**Mechanism of Action**	**Dose**	**References**
Murine Microglial N13 cells	Crude extracts	Expression of IL-1β, inducible nitric oxide synthetase, and Tumor necrosis factor-α is downregulated	10mg/ml	(54)
In Wister Rats, Carrageenan was used to induce paw edema.	Crude extracts	Less edema formation, neutralization of reactive oxidation species-induced lipid peroxidation, reduced activity of Glutathione, superoxide dismutase and catalase	400 mg/kg body weight	(31)
Caco-2 cells (Human Colon cancer cell lines)	Crude extracts	Reduced reactive species induced by Hydrogen peroxide, protection of cell from dysfunction barrier, lowering the depletion of IκBα	0.05 mg/mL	(53)
Inflammatory Response to carrageenan in Wister Rats	Isorhamnetin-3-O-glucosyl-rhamnosyl-rhamnoside Isorhamnetin-3-O-glucosyl-rhamnoside	Inhibited Cyclooxygenase-2 activity, reduced amounts of total leukocytes and edema formation, lower NO production, downregulated tumor necrosis factor-α and Interleukin-6	5 mg/kg body weight	(55)

Apart from its important antioxidant properties, thanks to molecules such as isorhamnetin derivatives, phenol compounds, and fractions of flavonoids (including rutin, ascorbic acid, betacyanins, and betalains) (21, 71), the medicinal plant OFI also contains other protective compounds, such as phenolic acid, vitamins C and E, and some non-nutrient materials. The in vitro lipid oxidation introduced in the erythrocytes by ethanol can be secured by using OFI fruit extract (72). The natural antioxidants present in OFI juice can help in saving and restoring the glutathione levels in the rat being given ethanol. This can be because of the inborn property of OFI to create a balance between the antioxidant defense and oxidant species (73,74).

The ascorbic acid present in OFI fruits has 68% antioxidant activity, while this antioxidant has less content in other species of fruits—for example, 9mg per 100g of fresh peaches, 7mg per 100g of fresh plums, and 10mg per 100g of fresh nectarines (66). The antioxidant effects of the OFI glycoprotein were studied in vitro, and it was reported that they do not have any cytotoxic effect; also, they help in protecting the liver cells by stopping the radical production of glucose/glucose oxidase (75). A study has been done in which OFI dried leaves have reduced the level of triglycerides and Low-Density Lipoprotein (LDL) cholesterol by the expeditious increase in the levels of High-Density Lipoprotein (HDL) cholesterol (76).

Diabetes

Type 2 Diabetes Mellitus (T2DM) is a significant global health concern, caused by different factors that include the patient's lifestyle or a presence of the disease in their family. The incidence of this disease is increasing day by day and it can affect different organs, such as the eyes, micro- and microvasculature, the nervous system, the kidneys, and the heart (77). The increasing incidence of T2DM can be explained by the metabolic syndrome, showing link between the changes in lipid and carbohydrate metabolism along with obesity and this disease. T2DM is a major public health concern, which can be overcome by the use of medicinal plants. For example, plants from the *Opuntia* family have experimental and clinical importance. The components of these species were tested temporarily on healthy volunteers, rabbits with hyperglycemia, patients with type II diabetes, and rabbits having alloxan-diabetes (78-84).

The anti-hyperinsulinemic and anti-hyperglycemic properties of *Opuntia* have been studied from the reports of animals, along with diabetes patients (85). The dietary fibers in the plant, like mucilage and pectin, can help in generating hypoglycemic mechanisms in the gut by increasing food viscosity and decreasing glucose absorption (86-88). It has also been hypothesized that the pancreatic beta cells can increase the release of insulin after the use of *Opuntia*, which has also resulted in an increase in the resynthesis of glycogen in healthy subjects after exercise (89, 90). A group of young, healthy volunteers, who had previously participated in strenuous exercise, were given an extract from the skin and cladode of an Opuntia ficus-indica fruit and were instructed to take it orally. As a consequence, the amount of glucose in their blood decreased, and this was made possible by an increase in the concentration of insulin in their serum (91).

Obesity

Obesity is a major problem worldwide, with an incidence rate of 40 million children and 500 million adults (92). The use of medicinal plants has emerged as a current subject in the context of addressing obesity. The genus *Opuntia* has its clinic pharmacological safety and efficacy, as in the past few years this plant has been used for treating obesity (56, 93,94). *Opuntia* extract has been studied to reduce the functioning of adipose tissues and their linked changes due to obesity. The OFI present in the diet can help in decreasing the level of cholesterol in murine plasma (95). The exact mechanism for the action of OFI against obesity is not clear. However, it can be hypothesized from different studies that the high amount of fibers present in this plant can act as a prebiotic for the microflora in the gut, which will help in avoiding obesity (96,97).

Cancer

The fruit, stems, seeds and roots of *Opuntia sp*. have been studied for their cytotoxic effects on cancer cell lines (98). Different species of *Opuntia* have been studied on different cell lines. The most affected cell lines are Caco2 (colon) and PC3 (prostate), but the HepG2 hepatic and MCF-7 mammary cell lines have shown less impact (74). The concentrated plant juice containing polyphenols has demonstrated cytotoxic properties against HT-29 colon cancer cell lines, while exhibiting minimal efficacy against the Caco2 cell lines. In contrast to this the natural extract from the peels and seeds of the plant has caused arrest of cell-cycle in the cells mentioned above (99). An in vitro and in vivo study has been done, in which OFI extract has resulted in a decreased proliferation of cells in bladder, cervical, and ovarian cancer cell lines. Additionally, the extract resulted in suppression of cancer growth in the in-vivo nude mice ovarian cancer model (100, 101). Betanin a betacyanin pigment, purified from OFI fruits, induces apoptosis in human chronic myeloid leukemia Cell line-K562 (102).

Neuronal diseases

The methanol extract from OFI fruit has been reported to treat the neuronal disease in mouse cortical cultures due to free radicals (103). The methanol extract from the plant stem was also helpful in the rat cortical cell cultures, as it decreased the oxidative injury caused by the xanthine (X) or xanthine oxidase (XO) and by H_2O_2 (104). A study reported that OFI administration for 7 days has resulted in increasing the phosphorylated extracellular signal-regulated kinase (pERK), the brain-derived neutrophic factor (BDNF), and the phosphorylated cAMP response element binding-protein (pCREB) in the hippocampal tissue. This resulted in increasing the long-term memory by the intake of OFI for a long time; the regulation of this mechanism is done by the survival of immature neurons and the brain-derived neurotrophic factor (BDNF), extracellular signal regulated kinase (ERK), and cAMP-response element-binding protein (CREB) (105).

The in vitro and in vivo study was done for the cerebral ischemia models having the neuronal injury. In this study, the methanol extract from OFI reduces the neuronal insult that occurs due to the increase in the peroxynitrite and microglial cells activity, as well as the inhibition of the production of nitric oxide (NO) (106,107). Another study has reported that the flavonoids extracted from OFI can be used as antidepressants (108).

Other diseases

Also renal diseases can be overcome by the help of OFI: a study reported that the flower of this plant can increase the natriuresis and diuresis (109). Another study on rats has reported that prickly pear flowers and cladode result in increasing the diuresis, but not specifically affecting the pattern of uric acid; which was more significant when the fruit of the plant was used for the treatment of the chronic state as well (110). Table 2 presents evidence supporting the therapeutic application of OFI in the management of chronic diseases.

Clinical Studies and Evidence

The physiological characteristics of *Opuntia sp* have been extensively studied via experimental and model organisms, as well as clinical studies conducted on humans. These studies have made it possible to investigate and define the therapeutic benefits of *Opuntia*-supplemented diets in a variety of chronic diseases (115). Currently, an insufficient amount of research has examined the possible impacts of OFI on lipid processing, glucose homeostasis, and oxidative stress-induced damage. These studies mostly include in vitro investigations; however, some also incorporated in vivo experimentation (19).

The impact of OFI on the regulation of blood glucose metabolism was investigated in mice for a duration of four weeks: after the conclusion of this time frame, there was a significant reduction observed in food consumption, as well as notable decreases in plasma glucose and insulin levels. In addition, it was observed through histological investigation that the pancreatic islets exhibited enhanced morphology in the mice subjected to OFI treatment (116).

In a rat model of acute inflammation, OFI-derived Ind was tested for protection. Injecting 0.2 mL carrageenan intrapleurally produced pleurisy. Exudates and inflammatory indicators were collected by euthanizing animals at 4h, 24h, and 48h. Different oral Ind doses (0.5, 1, and 2 μmol/kg) significantly reduced the pleural cavity exudate volume and the leukocyte count (95%). Similarly, the highest indicaxanthin dose showed reduced PGE2, NO, IL-1β, COX-2, iNOS, and TNF-α levels. This study revealed pigment properties that may increase well-being and minimize inflammation (117).

According to reported findings, the average body weight, total plasma cholesterol, and LDL in male albino and Wister rats showed a significant reduction after feeding them prickly pear seed oil: this is due to the presence of phytosterols (more specifically, vitamin E, αsitosterol and αcarotene), as it has also been observed in reducing lipoprotein level in total plasma (118).

The late maturity stage of *Opuntia* cladodes may result in the bioavailability of calcium: their consumption may thus prevent osteoporosis and other bone diseases (109). It has been previously documented that *Opuntia* exhibits a notable calcium content, estimated to be around 164 mg per 100 g

Table 2. Pharmacological effects of OFI on chronic diseases.

Chronic Diseases	Experimental evidence	References
Metabolic syndrome	Rabbits with alloxan diabetes and hyperglycemia. Reduced cholesterol and low-density lipoprotein cholesterol (LDL-C) levels in mice with hypercholesterolemia.	(79, 81, 84) (112)
Renal disease	Rats will have high level of natriuretic and diuresis. High level of potassium and sodium in the urine of rats, leading to antiuresis and diuresis.	(111) (101)
Inflammatory disease	In mice, the anti-inflammatory action was studied in chronic inflammatory model, resulting in stopping the release of a lysosomal enzyme beta-glucuronidase.	(113)
Neoplastic disease	A decrease in the proliferation has been studied in vitro, which resulted in the breakage of ADP ribose polymerase and the secretion of cytochrome c to cytosol from mitochondria.	(103)
Neuronal disease	In vitro inhibition of xanthine/xanthine oxidase (X/NO) in neurons for their protection. In vitro and in vivo models have shown the scavenging activity of peroxynitrite and microglial cells with the inhibition of NO production; as a result, there was an increased activity of cerebral ischemia.	(105, 106) (108)
Oxidative stress-related pathological processes	High scavenger activity in the erythrocytes of rat, helping to protect from the damage caused by ethanol. Inhibition of the activity of G/GO-induced radical production and help in liver cell protection.	(73, 75) (114)

of dried weight (119). Furthermore, a notable finding was made about the calcium concentration in *O. ficus-indica*: the calcium concentration in cladodes experiences a substantial increase of approximately 70% throughout the late maturity stages, as compared to cladodes in the early stage (120).

Conclusions and Future Prospects

The fibers, protective peptides, vitamins, and antioxidants of *Opuntia* have been studied in several researches: their biological effects have been studied in vitro, in animal models, and in clinical trials. These studies show that these compounds are antidiabetic, lipid-lowering, antiatherogenic, and may also decrease the growth of tumor cells. However, *Opuntia* needs further investigation to standardize and assure safety.

The interest in adopting alternative therapies shows a greater awareness and promotion of health and well-being among the public. Research on *OFIs* various qualities has garnered interest in functional food and nutraceuticals. According to previous studies, both phenolic and non-phenolic *Opuntia* chemicals have antioxidant, antibacterial, and other biological actions. More research is required to identify the optimal dosages of *Opuntia* compounds, both the ones that are largely known and also the newly found ones. Finding new uses for *Opuntia* needs advanced ways to analyze the chemical structure of possibly undiscovered molecules. It is an extremely versatile plant, since it also helps in dealing with chronic problems—including aging, diabetes, obesity, infectious diseases, metabolic syndromes, cardiovascular disorders, and neurological ailments. Scientific studies on the nutritional and therapeutic characteristics of plant specimens is gaining more and more academic attention, also thanks to its great adaptability to dry climates.

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Conflicts of interest statement

Authors declare no conflict of interest.

Abbreviations

Caco2	Colon Cancer Cell Line
COX	Cyclooxygenase
CVD	Cardiovascular Diseases
ECM	Extracellular Matrix
HepG2	Hepatoblastoma Cell Line
HT-29	Human Colorectal Adenocarcinoma Cell Line
IKK	IκB kinase
IL	Interleukins
iNOS	Inducible nitric oxide synthase
LPS	Lipopolysaccharide
LOX	Lipoxygenase
MCF7	Michigan Cancer Foundation
NF-KB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NO	Nitric Oxide
OFI	*Opuntia ficus-indica*
PC3	Prostate Cancer Cell Line
ROS	Reactive Oxygen Species
T2DM	Type 2 Diabetes Mellitus
TNF-α	Tumor necrosis factor-α

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Effects of Carob Extract on the Intestinal Microbiome and Glucose Metabolism: A Systematic Review and Meta-Analysis

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Abstract

The legume tree known as carob (*Ceratonia siliqua L.*) is indigenous to the Mediterranean area and over the centuries its pods had been traditionally used mostly as animal feed. However, it has gained great attention in human nutrition due to the molecular compounds it contains, which could offer many potential health benefits: for example, carob is renowned for its high content of fiber, vitamins, and minerals. Moreover, in traditional medicine it is credited with the ability to control glucose metabolism and gut microbiome. Modern science has also extensively acknowledged the numerous health advantages deriving from its consumption, including its anti-diabetic, anti-inflammatory, and antioxidant properties. Due to its abundant contents of pectin, gums, and polyphenols (such as pinitol), carob has garnered significant attention as a well-researched plant with remarkable therapeutic properties. Notably, carob is extensively used in the production of semi-finished pastry products, particularly in ice cream and other creams (especially as a substitute for cocoa/chocolate): these applications indeed facilitate the exploration of its positive effects on glucose metabolism.

Our study aimed at examining the effects of carob extract on intestinal microbiota and glucose metabolism. In this review, we conducted a thorough examination, comprising in vitro, in vivo, and clinical trials to appraise the consequences on human health of polyphenols and pectin from different carob species, including recently discovered ones with high polyphenol contents. Our goal was to learn more about the mechanisms through which carob extract can support a balanced gut flora and improve one's glucose metabolism. These results could influence the creation of novel functional foods and dietary supplements, to help with the management and prevention of chronic illnesses like diabetes and obesity. ***Clin Ter 2023; 174 Suppl. 2 (6):169-172 doi: 10.7417/CT.2023.2484***

Key words: *Carob, dietary supplement, functional food, glucose metabolism, intestinal microbiome*

Introduction

Carob Pod: A Center of Nutritional Components

Carob is a perennial plant of the Leguminosae family, typically measuring 8 to 16 meters in height, with strong branches and a thick trunk. Widely distributed across various regions of the Mediterranean basin, carob thrives even in conditions of high salinity and drought; moreover, its deep roots facilitate the absorption of carbon dioxide, making it also environmentally beneficial (1). Traditionally, as mentioned above, carobs were excluded from the human diet and were primarily employed as animal feed; however, carob fruits and/or pods are now used to create multiple foods and beverages, with many products—such as bakery items, pasta, milk alternatives, etc.—progressively incorporating carob pods. It is commonly processed into powder, flour, and syrup, which are used in the production of confections, as well as chocolate or cocoa alternatives.

The primary yield of this tree is the carob pod, also known as the "locust bean," which serves as an edible bean. Made of 90% pulp and 10% seeds (2), the pod exhibits an elongated shape, with varying lengths and a brownish-lined outer surface (3). The pulp can be further divided into two parts: the rough external layer, called the pericarp, and the smooth inner layer, known as the mesocarp (4). Carob tree leaves possess a thick epidermis, rich in phenolic compounds.

The carob pod naturally contains a high sugar content (around 45 to 55%), which is composed as follows: over 90% sucrose, 2 to 4% glucose, and a minute amount of fructose (5). It also contains negligible fats, and its protein content is around 7%. Carob pods are rich in insoluble fibers (cellulose, hemicellulose, lignin) that help to counteract high glucose levels. They also contain phenolic compounds—such as

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phenolic acid, flavonoids, and gallotannins—with cholesterol-lowering effects, which are beneficial for managing diabetes (6). Carob pods also provide vitamins and minerals (like calcium, potassium, magnesium, and phosphorus) (7). Carob seeds contain various phenolic chemicals, including flavonols and tannins.

Pectin, a heteropolysaccharide found in the cell walls of terrestrial plants, is abundantly found in carobs. It primarily consists of galacturonic acid, a naturally occurring sugar that is derived from galactose. Pectin has been shown to enhance the oxidation of lipids as well as cholesterol metabolism; it is also found to reduce postprandial acylated ghrelin (8). Pectin supplementation improves glucose tolerance and alters the response of postprandial blood glucose levels (9).

Polyphenols and Their Extraction Methods from the Carob Pod

To extract crucial nutrients from the pods of carob trees, they are first dried, so that moisture is reduced. Subsequently, pulp and seeds are separated from one another. The pulp, which is rich in sugar, fiber, and antioxidants, can be used for industrial food production (2), usually following this process: first, the pulp is ground and roasted; afterwards, it is refined and transformed into a powder. To produce the carob syrup, the ground pulp is immersed in water, drained, and finally boiled.

Carob pods are a good source of fiber, calcium, and other nutrients. They can be consumed raw, roasted, or powdered; carob powder can be used in recipes in place of cocoa powder, or it can be mixed in with yoghurt, muesli, or smoothies. In addition to that, carob pods contain abundant quantities of the plant chemicals known as polyphenols (such as tannins, flavonoids, and phenolic acids), which are renowned for their anti-inflammatory and antioxidant qualities. Researchers have shown that consuming carob pods polyphenols leads to a wide range of health advantages, such as lowering the risk of diabetes, cancer, and heart disease. Polyphenols' antioxidant properties and their ability to stop chronic illnesses have been the subject of many researches, according to which polyphenols from carob extracts play a role in several signaling pathways that may be beneficial to human health. Carob pod polyphenols have potent anti-free radical properties, which help to counteract the damage from oxidative stress and to shield cells from injury.

One interesting carob-derived polyphenol is pinitol, which can be obtained by following an easy protocol: a dilution of carob syrup is prepared to eliminate the glucose components through the farming of microorganisms; then, the microbes' cells are eliminated either by filtration or centrifugation. Pinitol is then separated, using a column of concentrated activated carbon to form crystals. Mass spectrometry enables the quantification of pinitol in carob syrup (10).

Additionally, carob pod's polyphenols have demonstrated promising anti-inflammatory benefits. They have the power to control inflammatory pathways and stop the body from producing pro-inflammatory chemicals. This anti-inflammatory property adds to the carob pod's potential for reducing inflammation and enhancing general health. Additionally, the polyphenols in carob pods were shown to possess antibacterial characteristics, making them useful in preventing the growth of specific bacterial strains and in promoting a balanced gut flora (11).

The Physiologic Beneficial Effects of D-Pinitol

Carob pods contain the naturally occurring substance D-pinitol; in fact, carob is the sole commercially viable source of this molecule. It is a new nutritional supplement that shows promise for enhancing overall health and well-being, thanks to its variety of health beneficial effects on the body, including anti-inflammatory, antioxidant, and hypolipidemic properties, making it a safe and efficient substance, possible to be acquired both from carob pods and as a food supplement. It has been demonstrated to have antidiabetic, anti-inflammatory, and antioxidant properties: heart disease, cancer, and Alzheimer's disease are just a few of the chronic conditions that D-pinitol's properties may help to prevent.

D-pinitol is a form of phytosterol, which compose a group of plant-derived substances with structures resembling those of cholesterol: this way, they prevent the absorption of cholesterol from the stomach, aiding in lowering blood cholesterol levels. D-pinitol is one of the best phytosterols for decreasing cholesterol levels; moreover, it also exhibits insulin-mimicking effects by independently reducing glucose levels. D-pinitol improves the function of glucose transporter 4 (GLUT4), responsible for glucose transport to adipose tissues and muscles and regulated through the PI3K/Akt signaling pathway (12, 13, 14).

Sivakumar et al. conducted research indicating that D-pinitol acts as an antioxidant compound with the capacity to scavenge free radicals, thereby reducing oxidative stress associated with many health conditions (15). Studies have also revealed the potential of D-pinitol to inhibit the progression of cancers, both in vivo and in vitro (16). Experimental findings have also shown that the nuclear factor kappa B pathway can be blocked by D-pinitol (17), which serves as an operative approach for inhibiting cell invasion induced by the Tumor necrosis factor (TNF) and downregulating genes that play a role in increasing tumor metastases (18).

Additionally, studies have shown that D-pinitol contains antioxidant characteristics that can efficiently scavenge free radicals and reduce oxidative stress in the body. D-pinitol may aid in the prevention and treatment of numerous illnesses linked to cellular damage by lowering oxidative stress. Furthermore, D-pinitol exhibits activity to protect the liver, and it can also protect the pancreatic tissue from oxidative stress. Additionally, it has been demonstrated that D-pinitol can reduce the activity of inflammatory cytokines, like TNF-a (19). It can also function in suppressing the activities of the immune system by regulating the cytokine balance of Th1/Th2 (20).

In Vitro and In Vivo Studies on Carob Pod Polyphenols

Given the ability of carob pod polyphenols to reduce glucose levels, in vitro experimental studies have aimed at revealing their anti-obesity activities. Fujita et al. revealed that carob pod polyphenols play a role in preventing the

differentiation of adipocytes by controlling C/EBPB at the post-transcriptional level (21). These polyphenols also exhibit the capacity to reduce the activity of αglucosidase (22). Furthermore, in vitro tests on mature 3T3-L1 adipocytes have shown that carob pod polyphenols have anti-inflammatory effects as well (23).

Furthermore, the in vitro studies found that carob pod polyphenols also have antioxidant capacities (24). An experimental study showed that supplementation with carob fruit extract aided in losing weight by reducing the levels of IL-6 in plasma and increasing levels of adiponectin in mice (25). The post-transcriptional control of C/EBPB allowed carob pod polyphenols to prevent adipocyte differentiation. They also exhibited the ability to reduce the activity of αglucosidase in vitro. Other studies showed that carob pod polyphenols prevented an increase in adipose tissue weight in mice with diet-induced obesity (21), and supplementation with carob pod extract improved glucose tolerance and reduced glucose levels in mice (26); also, carob leaf extract infusion mitigated inflammation by reducing pro-inflammatory cytokine levels in obese mice (27).

For example, carob fruit extract was administered to rats with type 2 diabetes, leading to a notable improvement in their gut microbiota dysbiosis (28). Macho-Gonzalez et al. found that carob fruit extract reduced blood glucose levels in rats, indicating its potential for treating hyperglycemic conditions (22). Other studies show that the histological pancreatic sections of diabetic rats that were given carob extracts showed the least damage of αcells compared to diabetic controls (29), and that carob powder-based snacks exhibited an anti-hypertensive effect in rats with metabolic syndrome, effectively addressing complications associated with metabolic syndrome (23).

Human studies on Carob pod polyphenols

Numerous human studies have been conducted to examine the effects of carob pod polyphenols (CPPs): these compounds can help people with hypertension to lower their blood pressure, and they may also aid in enhancing type 2 diabetes patients' ability to control their blood sugar. Moreover, CPPs have also been demonstrated to possess antioxidant and anti-inflammatory properties (30).

The potential of CPPs in controlling blood glucose levels and diabetes is another interesting area of research in human investigations. According to research, the anti-diabetic effects of these polyphenols might be due to them modifying insulin sensitivity and glucose metabolism. CPPs have been shown in clinical trials to promote overall glycemic control, reduce insulin resistance, and improve postprandial blood glucose management in people with diabetes or impaired glucose tolerance (31).

Additionally, research on the benefits of carob pod polyphenols for digestive health has yielded encouraging results. According to human research, these polyphenols have prebiotic qualities that support the development of good gut bacteria and regulate the composition of the gut microbiota. Additionally, it has been shown that CPP have anti-inflammatory properties in the digestive system, which may help to reduce the symptoms of gastrointestinal illnesses and promote overall gut health (32).

Materials and Methods

This is a "qualitative review" of the physiologic beneficial effects of carobs. All the information used for the preparation of this document is based on data from original documents and reviews.

We selected the most relevant studies on polyphenols based on the physiologic beneficial effects of carob. To categorize the many carob species and identify those with high polyphenol concentrations, the corpus of current knowledge was thoroughly reviewed and an electronic search in the PubMed and Scopus databases was performed.

Conclusion

In conclusion, our systematic review and meta-analysis focused on the effects of carob extract on the intestinal microbiome and glucose metabolism. Carob extract has been shown to have beneficial effects on the intestinal microbiome and glucose metabolism in both in vitro and in vivo studies. Through an extensive examination of in vitro, in vivo, and clinical trials, we have summarized the main physiological activity of carob and its role in human health. Carob extract, rich in pectin, gums, and polyphenols such as D-pinitol, has demonstrated anti-diabetic, anti-inflammatory, and antioxidant properties. It exhibits the potential to support balanced gut flora, improve glucose metabolism, and aid in the management and prevention of chronic illnesses like diabetes and obesity. These findings highlight the importance of carob as a potential source for developing functional foods and dietary supplements with therapeutic properties.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review

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Nutrigenomics: SNPs correlated to vitamins' deficiencies

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Abstract

Nutrients can influence the physiological processes in the body by interacting with molecular systems. Including nutrigenetics and nutrigenomics, nutritional genomics focuses on how bio-active food components interact with the genome. The purpose of this study is to clarify how nutrigenomics and vitamin dietary deficits relate to one another. Food tolerances among human sub-populations are known to vary due to genetic variation, which may also affect dietary needs. This raises the prospect of tailoring a person's nutritional intake for optimum health and illness prevention, based on their unique genome. To better understand the interplay between genes and nutrients and to plan tailored weight loss, nutrigenetic testing may soon become a key approach. ***Clin Ter 2023; 174 Suppl. 2 (6):173-182 doi: 10.7417/CT.2023.2485***

Key words: *Nutrients, SNPs, nutrigenomics, biomarkers, genes, vitamin A, vitamin B6, vitamin B9, vitamin B12, vitamin C, vitamin D, vitamin E, choline*

Introduction

Diet has significantly altered human metabolic capabilities throughout human development, which has facilitated the rise of contemporary disorders. From an evolutionary perspective, nutrition is a limiting element that puts selective strain on a population, just like other variables in the environment. Selection will be made against certain genotypes when nutritional needs in an individual are not met (1). A population's genotypes vary in their nutrient requirements. However, until these requirements—such as the need for additional calories from carbs and dietary fat—are addressed, the gene that transmits the high dietary requirement will persist in the population. This might apply to genes linked to diabetes and obesity (2).

In the developing discipline of nutrigenomics, cutting-edge genomics methods are used to examine how nutrients affect the genome and gene expression, as well as how genetic variations affect nutrient consumption. To characterize the relationship between nutrients and genes, the term "nutrigenomics" was coined. Physiology, biochemistry, metabolomics, proteomics, transcriptomics, and bioinformatics are all connected to genetics through nutrigenomics (3). Numerous genetic variations have been shown to affect the way proteins are built and function. The study of how dietary factors differently affect people, depending on their genetic makeup, is known as nutrigenetics, and it is a subfield of dietary genome research. Nutrigenomics investigates how dietary factors interact with the genome to regulate modifications to proteins and other metabolic functions (4).

A variety of dietary components serve as cofactors or substrates in metabolic pathways and are essential for DNA metabolism and repair, but there is much less information available about the effects of cofactor on the accuracy of DNA replication and repair. The genotype-dependent response to a particular nutrient must also be taken into consideration, even if certain nutrients can influence how a phenotype develops (5). The importance of genetic coding for assessing genome durability and related health impacts like cancer, degenerative diseases, and postnatal anomalies is widely acknowledged. It is clear that complicated chronic illnesses can emerge due to both ecological and hereditary factors (6). The "fetal basis of adult disease" or "early origins hypothesis" postulates that nutrition and other environmental factors during pregnancy and the early postnatal period have an impact on gene activity and cellular flexibility, which can alter vulnerability to mature illnesses (7).

Vitamins, their role, and effect of vitamin deficiency

Vitamins are necessary chemical substances for the human body to ensure appropriate physiological function and overall health. They operate as coenzymes or as precursors for enzymes that assist reactions within the body, playing important roles in a variety of metabolic processes (8). Thiamine is necessary for the metabolism of food into energy, optimal neuronal function, and preservation of the cardiovascular system. The immune system, the creation of neurotransmitters, and the metabolism of proteins all require

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pyridoxine (9). B12 is essential for synthesizing DNA, producing red blood cells, and maintaining healthy nerves: its deficiency might lead to pernicious anemia, nerve damage, and cognitive impairment. The immune system, eyesight, and cell differentiation all depend on vitamin A; among the symptoms of its deficiency are night blindness, dry skin, and an increased risk of infections (10). Antioxidants like vitamin E shield cells from harm, and its absence, although uncommon, can cause neurological problems and muscle weakness. Bone health and blood clotting depend on vitamin K, whose deficiency can decrease blood coagulation and thus raise the risk of bleeding. The immune system, bone health, and calcium absorption all depend on vitamin D. Vitamin deficiency can be avoided by eating a balanced diet that is high in fruits, vegetables, whole grains, and a variety of foods, while malnutrition, restrictive diets, digestive issues, and a few medical diseases can all raise the risk of deficiency, though. Consult a healthcare provider as soon as you suspect a deficiency for accurate diagnosis and effective therapy (11).

Nutrigenetics

Nutrigenetics studies the impact of genetic diversity on nutrient responses and function. Although they are closely linked, it is not the same thing as nutrigenomics (5). Numerous chronic diseases can be predicted using nutrigenetic research, and these conditions may be avoided or treated more effectively by using individualized nutritional management. The majority of nutrigenetic experiments investigate how various polymorphisms influence alterations in eating patterns (5).

Nutrigenomics

The goal of the emerging area of nutrigenomics, which makes use of the most cutting-edge genomics technology available, is to investigate how vitamins affect the genome and gene expression, as well as how genetic variations affect nutrient consumption (12). The area of nutritional research known as "nutrigenomics" focuses on using molecular methods to examine, evaluate, and comprehend the physiological responses of certain populations or individuals to different diets (2). These gene-nutrient interactions depend on the ability of specific nutrients to interact with transcription factors, which in turn regulates RNA polymerase's attraction to gene promoter areas and the quantity of transcripts produced (3).

Single nucleotide polymorphisms (SNPs)

The most prevalent sort of variation in human DNA is single nucleotide polymorphisms (SNPs). In the last two decades, nutrigenomics studies identified phenotypes of SNPs between healthy and micronutrient-deficient populations (13). This correlations usually refer only to nutritional deficiencies. Thus, more research and human studies are needed to determine what function, if any, these SNPs have in developing specific nutrient deficits or other physiological responses (14).

Choline

An important vitamin, called choline, is crucial for several bodily physiological processes. Many foods, both from animal and plant sources, contain choline, but among its main sources are egg yolk, chicken, fish, and dairy products (15). In comparison to other vitamin deficiencies, choline insufficiency is less researched and understood. For people who struggle to get enough choline from their food alone or have particular health concerns that call for higher choline consumption, dietary supplements can be used to have an extra source of choline (16). However, it's crucial to remember that the majority of people can satisfy their choline requirements by eating a well-balanced diet. The undesirable consequences of excessive choline intake may include a fishy body odor or digestive problems (17). (Table 1)

The phosphatidylethanolamine N-methyl transferase (PEMT) gene's particular genetic variations, or single nucleotide polymorphisms (SNPs), are described in Table 1, which includes information on two SNPs (rs12325817 and rs7946) as well as the associated genes, polymorphism functions, and alleles (23). The methylation pathway, which is essential for

Table 1. SNPs related to choline and their effects.

Choline					
RsID	**Gene**	**Polymorphism function**	**Alleles**	**wt/mt**	**Reference**
rs12325817	PEMT	Increased risk of organ dysfunction with low choline diet; lower betaine levels with inadequate choline intake	G/G	mt/mt	(18-20)
		Increased risk of organ dysfunction with low choline diet	C/G	wt/mt	
		Typical	C/C	wt/wt	
rs7946	PEMT	Decreased enzyme activity	T/T	mt/mt	(21-23)
		Somewhat decreased enzyme activity	C/T	wt/mt	
		Typical	C/C	wt/wt	

the manufacture of phosphatidylcholine, is catalyzed by the PEMT gene. The structure of cell membranes is crucially maintained by phosphatidylcholine. People who consume a choline-deficient diet are at an increased risk of developing organ malfunction due to the specific polymorphism indicated by the rs12325817 SNP (24).

Vitamin B6

One of the B-complex vitamins, vitamin B6, often referred to as pyridoxine, is essential for several bodily metabolic activities. As a coenzyme, it speeds up numerous enzymatic processes that are necessary for the metabolism of proteins and the creation of neurotransmitters like serotonin and dopamine. Many foods, both plant-based and animal-based, contain vitamin B6 (25). The finest food sources of vitamin B6 include vegetables, whole grains, legumes, chicken, fish, organ meats, nuts, seeds, and legume-based products. A lack of vitamin B6 can cause neurological issues, skin disorders, irritability and sadness, anemia, weakness, and exhaustion (26). There are vitamin B6 supplements available for people who might have trouble getting enough of it through diet alone, or have particular medical conditions that call for higher B6 intake. The most common forms of these supplements are pyridoxine hydrochloride and pyridoxal-5'-phosphate (27).

SNPs that affect how vitamin B6 is metabolized are included in Table 2, which contains information on two SNPs (rs4654748 and rs5742905) as well as the genes, gene functions, polymorphism functions, and alleles that are connected to them (31). Alkaline phosphatase, an enzyme involved in the metabolism of several phosphate compounds, is represented by the gene ALPL in Table 2. It is essential for adaptive thermogenesis and skeletal mineralization. Lower levels of vitamin B6 are linked to the particular polymorphism indicated by rs4654748 (32). The enzyme known as cystathionine beta-synthase, which is involved in the metabolism and detoxifying processes of cysteine, is encoded for by CBS. An increased risk of excessive homocysteine levels is linked to the particular polymorphism indicated by rs5742905; however, taking extra vitamin B6 can lessen or mitigate this risk.

Vitamin B9

The vital water-soluble vitamin B9, sometimes referred to as folate or folic acid, is involved in a number of key bodily processes. Its main function is to function as a coenzyme in DNA synthesis, cell division, and red blood cell production. Numerous foods naturally contain folate, and many nations also fortify some meals with folic acid to help prevent deficiencies (33). Leafy green vegetables, legumes, citrus fruits, avocados, fortified grains, and liver are a few examples of foods high in folate (34). Due to its critical function in cell division and DNA synthesis, vitamin B9 insufficiency, also known as folate deficiency, can cause a number of health issues. Anemia, neural tube abnormalities, digestive problems, and mood disorders are typical signs of folate insufficiency. Supplements containing folic acid are frequently used to prevent and treat folate deficiency, particularly in pregnant women and others who may have problems getting enough folate through their diet. Prenatal vitamins and over-the-counter folic acid supplements are both readily available (35).

Table 3 offers details on a particular genetic variant (SNP) connected to folate (vitamin B9) metabolism; it also contains information about the MTHFR gene, the SNP rs1801133, the gene's related polymorphism, and the rele-

Table 2. SNPs related to Vitamin B6 and their effects.

Vitamin B6					
RsID	**Gene**	**Polymorphism function**	**Alleles**	**wt/mt**	**Reference**
rs4654748	ALPL	Lower vitamin B6 concentrations	C/C	mt/mt	(28)
		Slightly lower vitamin B6	C/T	wt/mt	
		Typical	T/T	wt/wt	
rs5742905	CBS	Risk of increased homocysteine, responsive to vitamin B6	G/G	mt/mt	(29, 30)
		Risk of increased homocysteine, responsive to vitamin B6	A/G	wt/mt	
		Typical	A/A	wt/wt	

Table 3. SNPs related to Vitamin B9 and their effects.

Vitamin B9					
RsID	**Gene**	**Polymorphism function**	**Alleles**	**wt/mt**	**Reference**
rs1801133	MTHFR	Enzyme function decreased by 70-80%	A/A	mt/mt	(36)
		Enzyme function decreased by 40%	A/G	wt/mt	
		Typical	G/G	wt/wt	

vant alleles. the MTHFR gene codes for the enzyme called methylenetetrahydrofolate reductase (37). It is important to note that the decreased enzyme activity brought on by this polymorphism might result in higher homocysteine levels and may be linked to a number of health issues, including cardiovascular disease and neural tube abnormalities (38).

Vitamin B12

Cobalamin, generally known as vitamin B12, is a water-soluble vitamin that is essential for several bodily physiological processes. It plays a major role in DNA synthesis, neuron function, and red blood cells' production. Foods derived from animals—such as meat, fish, shellfish, dairy products, and eggs—naturally contain vitamin B12 (39). A lack of this vitamin can cause a variety of health issues, with mild to severe symptoms. Common signs include weakness and exhaustion, anemia, difficulty walking, painful lips, memory loss and cognitive impairments, pale complexion, and stomach issues (40). Vitamin B12 deficiency can be treated or prevented with the help of dietary supplements, especially in people who may have trouble absorbing the vitamin through food sources. There are several ways to get vitamin B12 supplements, including oral tablets, lozenges, injections, and sublingual.

The genetic variations (SNPs) that affect how vitamin B12 is metabolized are listed in **Table 4**, which contains information on the SNPs rs602662, rs492602, rs1801222, and rs162036, as well as information on the genes, polymorphisms, and linked alleles that each SNP is associated with. The gene FUT2 codes for an enzyme that alters the glycan chains of glycolipids and glycoproteins present on cell surfaces. The specific polymorphism rs602662 is only related to the risk of low serum vitamin B12 levels when the diet is insufficient in bioavailable sources of vitamin B12. The particular polymorphism represented by rs492602, namely the G allele, is associated with lower levels of vitamin B12 in the body. The gene CUBN encodes for an endocytic receptor, specifically one that facilitates vitamin absorption. The G allele of the specific polymorphism denoted by rs1801222 is associated with elevated B12 levels in the body. MTRR regulates insulin secretion and has a relationship to ion channel genes. The amount of vitamin B12 that is absorbed or used by the body may vary depending on these genetic differences. Certain genotypes may increase the risk of low levels of vitamin B12 or alter the vitamin's blood level (45).

Vitamin E

The body needs vitamin E, a fat-soluble antioxidant, to function properly. Its main function is to shield cells from free radical damage. Free radicals are extremely reactive chemicals that can destroy cells and cause a number of health problems. Many foods contain vitamin E, although plant-based foods tend to be the best sources. Fortified cereals, avocado, kiwifruit, broccoli, and fortified cereals are some of the top food sources of vitamin E (46). Muscle weakness, nerve damage, eye issues, trouble walking and coordinating motions, and anemia are all signs of vitamin E insufficiency. Getting enough vitamin E from dietary sources should be sufficient for most healthy people who follow a balanced diet. There are several types of vitamin E supplements, including capsules, soft gels, and oils (47).

Table 4. SNPs related to vitamin B12 and their effects

Vitamin B12					
RsID	**Gene**	**Polymorphism function**	**Alleles**	**wt/mt**	**Reference**
rs602662	FUT2	Greatest risk for low serum vitamin B12 levels, but only when the diet is low in bioavailable sources of vitamin B12	G/G	mt/mt	(41)
		Greater risk for low serum vitamin B12 levels, but only when the diet is low in bioavailable sources of vitamin B12	G/A	wt/mt	
		Typical	A/A	wt/wt	
rs492602	FUT2	Lower vitamin B12 levels	G/G	mt/mt	(42)
		Lower vitamin B12 levels	G/A	wt/mt	
		Typical	A/A	wt/wt	
rs1801222	CUBN	Lower vitamin B12 concentrations	A/A	mt/mt	(43,44)
		Somewhat lower vitamin B12 concentrations	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs162036	MTRR	Decrease in enzyme activity with potential negative impact on vitamin B12 concentration	G/G	mt/mt	(43,44)
		Partial decrease in enzyme activity with potential negative impact on vitamin B12 concentration	A/G	wt/mt	
		Typical	A/A	wt/wt	

Table 5. SNPs related to vitamin E and their effects.

Vitamin E					
RsID	**Gene**	**Polymorphism function**	**Alleles**	**wt/mt**	**Reference**
rs11057830	SCARB1	Lower plasma vitamin E concentration	A/A	mt/mt	(47, 48)
		Somewhat lower plasma vitamin E concentration	G/A	wt/mt	
		Typical	G/G	wt/wt	
rs1527479	CD36	Lower plasma vitamin E concentration	A/A	mt/mt	(49)
		Somewhat lower plasma vitamin E concentration	G/A	wt/mt	
		Typical	G/G	wt/wt	
rs2108622	CYP4F2	Lower plasma vitamin E concentration	T/T	mt/mt	(49,50)
		Somewhat lower plasma vitamin E concentration	C/T	wt/mt	
		Typical	C/C	wt/wt	

The information about three genetic variations (rsID) and their relation to plasma vitamin E content is presented in Table 5. Each variant has a unique allelic combination and is linked to a particular gene. It serves as a high-density lipoprotein receptor. Lower levels of plasma vitamin E are linked to the A allele. This genotype results in reduced plasma vitamin E concentrations in the individuals. It makes it easier for fatty acids to pass through cell membranes. Lower levels of plasma vitamin E are linked to the A allele. This genotype results in reduced plasma vitamin E concentrations in the individuals. When compared to genotypes with normal plasma vitamin E content, individuals presenting this genotype had lower levels of vitamin E. It is involved in the metabolism of xenobiotics (foreign chemicals) and fatty acids. Variations in the amount of vitamin E in the blood are linked to the T allele. Individuals with this genotype have varying blood levels of vitamin E. The amounts of vitamin E in the blood may vary depending on genotype, with some alleles being linked to higher or lower levels (51).

Vitamin A

A vital component of sustaining vision, bolstering the immune system, and encouraging healthy skin and mucous membranes is the fat-soluble vitamin known as vitamin A. Both animal-based foods—such as liver and organ meats, fish, eggs, dairy products—and plant-based ones—like carrots, sweet potatoes, mangoes, and apricots—contain preformed vitamin A. Night blindness is among the signs of vitamin A insufficiency, along with dry skin, increased susceptibility to infections, and wound healing problems (52). Vitamin A supplements can be helpful for people who have trouble getting enough vitamin A through their meals or who are at risk of insufficiency. Several types of vitamin A supplements are available, including retinyl palmitate and beta-carotene (53).

Table 6 gives details on three genetic variations (rsID) and how they affect how beta-carotene is metabolized and transformed into vitamin A. Each variant has a unique allelic combination and is linked to a particular gene. Encodes a significant enzyme, necessary for the conversion of beta-carotene to vitamin A. In order to create two retinal molecules, it catalyzes the oxidative cleavage of beta-carotene. The G allele is linked to decreased beta-carotene conversion, which raises the amounts of beta-carotene in the blood. This genotype results in decreased beta-carotene conversion, which raises the amounts of beta-carotene in the blood. Individuals with this genotype and those with a normal genotype have somewhat decreased beta-carotene conversion. This gene encodes for a crucial enzyme, necessary for the conversion of beta-carotene to vitamin A. The T allele is linked to lower beta-carotene conversion and a higher risk of developing atherosclerosis when eating poorly. The metabolism of lycopene may also be impacted This gene encodes for a crucial enzyme, necessary for the conversion of beta-carotene to vitamin A. The T allele is linked to lower levels of lutein (another carotenoid) and impaired beta-carotene conversion. The efficiency of beta-carotene conversion may vary depending on genotype, which could alter levels of circulating beta-carotene and possibly other carotenoid levels, like lutein and lycopene (56).

Vitamin D

A vital nutrient, vitamin D performs a number of vital functions in the body. One of its main tasks is helping to control the absorption of calcium and phosphorus, which are essential for keeping strong bones and teeth. Fatty fish, cod liver oil, egg yolks, fortified dairy products or plant-based milk alternatives, and fortified morning cereals are a few food sources of vitamin D. Bone discomfort, soft bones, an increased risk of fractures, delayed wound healing, and diminished immunological function are some frequent signs of vitamin D deficiency [32]. If dietary consumption and sun exposure are insufficient to maintain optimal vitamin D levels, it may be advised to take dietary supplements. Supplemental vitamin D is frequently used to treat or prevent vitamin D insufficiency. They are frequently prescribed to people who don't get much sun exposure, like those who live in northern latitudes, elderly people who spend less time outside, people with darker skin, and people with illnesses that make it difficult for the body to absorb fat (57,58).

Table 6. SNPs related to vitamin A and their effects.

Vitamin A					
RsID	**Gene**	**Polymorphism function**	**Alleles**	**wt/mt**	**Reference**
rs6564851	BCO1	Decreased beta-carotene conversion	G/G	mt/mt	(54)
		Decreased beta-carotene conversion	G/T	wt/mt	
		Typical	T/T	wt/wt	
rs12934922	BCO1	Decreased beta-carotene conversion; may affect lycopene also	T/T	mt/mt	(1,55)
		Decreased beta-carotene conversion	A/T	wt/mt	
		Typical	A/A	wt/wt	
rs7501331	BCO1	Decreased beta-carotene conversion; lower lutein levels; may affect lycopene	T/T	mt/mt	(1,56)
		Decreased beta-carotene conversion	C/T	wt/mt	
		Typical	C/C	wt/wt	

Table 7. SNPs related to vitamin D and their effects.

Vitamin D					
RsID	**Gene**	**Polymorphism function**	**Alleles**	**wt/mt**	**Reference**
rs4588	GC	Lower 25-hydroxyvitamin D (main circulating form) levels	A/A	mt/mt	(59, 60)
		Somewhat lower 25-hydroxyvitamin D (main circulating form) levels	A/C	wt/mt	
		Typical	C/C	wt/wt	
rs2282679	GC	Decreased vitamin D levels	G/G	mt/mt	(61,62)
		Somewhat decreased vitamin D levels	G/T	wt/mt	
		Typical	T/T	wt/wt	
rs7041	GC	Decreased vitamin D levels	A/A	mt/mt	(63)
		Decreased vitamin D levels	A/C	wt/mt	
		Typical	C/C	wt/wt	
rs12794714	CYP2R1	Lower vitamin D levels	A/A	mt/mt	(64)
		Somewhat lower vitamin D levels	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs10741657	CYP2R1	Possible vitamin D insufficiency or deficiency	G/G	mt/mt	(65)
		Possible vitamin D insufficiency or deficiency	A/G	wt/mt	
		Typical	A/A	wt/wt	
rs2228570	VDR	Carrier of Fok1 variants; possibly decreased vitamin D levels	G/G	mt/mt	(66, 67, 68)
		Typical	A/G	wt/mt	
		Typical	G/G	wt/wt	

The information regarding various genetic variations (rsID) and their relationship to vitamin D levels is included in Table 7. Each variant has a unique allelic combination and is linked to a particular gene. Target tissues receive the vitamin D and its plasma metabolites once it binds to them. Lower 25(OH) D levels, which are a measure of vitamin D status, are linked to the A allele. Therefore, those who have this genotype have lower levels of 25(OH) D. In other words, those with the A/A genotype for the rs4588 variant of the GC gene have lower levels of 25(OH) D, a measure of their vitamin D status. This shows that this genetic variation may affect vitamin D transport or metabolism, resulting in reduced amounts of the active form of vitamin D in the blood (66).

Variant rs2282679; Gene: GC

Target tissues receive vitamin D and its plasma metabolites once it binds to them. Serum vitamin D levels are known to be lower in people with the G allele genotype. The levels

of total serum vitamin D are a little bit lower in all of the people with this genotype. As a result, those with the G/G genotype for the rs2282679 variant of the GC gene have decreased serum vitamin D levels. Similar to this, people with the G/T genotype have somewhat lower amounts of total serum vitamin D. This implies that this genetic variation may impact vitamin D binding or transport, resulting in lower levels of vitamin D circulating in the blood (67).

Variant rs7041; Gene: GC

Lower levels of serum vitamin D are found in people with the A/A genotype for the rs7041 variant of the GC gene. Similar to this, those with the A/C genotype have somewhat lower levels of vitamin D. This shows that this genetic variation may affect how vitamin D is bound or transported, resulting in lower quantities of vitamin D circulating in the blood (68).

Variant rs12794714; Gene: CYP2R1

Lower amounts of vitamin D are found in people with the rs12794714 variant of the CYP2R1 gene, which has an A/A genotype. A/G genotype carriers have an intermediate phenotype, which means that their levels of vitamin D are in between those of A/A and G/G genotype carriers. This shows that this genetic variation may have an impact on the CYP2R1 enzyme's function or efficiency, altering how vitamin D is converted into its active form and ultimately affecting vitamin D levels (69).

Variant rs10741657; Gene: CYP2R1

Individuals with the G/G genotype for the rs10741657 variant of the CYP2R1 gene are more likely to suffer from vitamin D deficiency or insufficiency, similarly to those with the A/G genotype. This shows that this genetic variation may affect the CYP2R1 enzyme's performance or activity, which could lower the conversion of vitamin D into its active form and increase the risk of low vitamin D levels.

Variant rs2228570; Gene: VDR

Individuals who carry FokI mutation have the G/G genotype for the rs2228570 variant of the VDR gene; as a result, the levels of vitamin D may decline. These people might also be more vulnerable to fractures, malignant melanoma, and dengue fever. Normal vitamin D levels are present in people with the A/G genotype and in people with the A/A genotype. This shows that the VDR protein's activity or function, which can affect vitamin D levels and potentially contribute to some health problems associated with vitamin D shortage, may be impacted by this genetic variant (70).

Vitamin C

Ascorbic acid, another name for vitamin C, is a water-soluble vitamin that is essential for many biological processes, such as the development, growth, and repair of body tissues. For example, the creation of collagen—a protein that serves as the building block of connective tissues in the skin, bones, and blood vessels—depends on vitamin C. Among the foods that are high in vitamin C are citrus fruits, berries, kiwis, mangoes, red and green bell peppers, tomatoes, spinach, and guavas (71). A lack of vitamin C can cause scurvy, a disorder with the following symptoms: anemia; weariness; bleeding gums and loose teeth; slow wound healing; dry, rough, and scaly skin; swelling and coloring of the skin; and depression. Vitamin C insufficiency can be prevented or treated with dietary supplementation. Supplements might be a helpful choice for people who cannot get enough vitamin C from their diet or have certain medical problems that prevent optimal absorption (72).

The information about two genetic variations (rsID) and their relationship to vitamin C levels is provided in Table 8. Each variation is linked to a unique gene that is involved in the transportation of vitamin C.

Variant rs33972313; Gene: SLC23A1

In comparison to those with the C/T or C/C genotypes, plasma vitamin C concentrations are lower in people with the T/T genotype for the rs33972313 variation of the SLC23A1 gene. This implies that this genetic variation may affect the effectiveness or uptake of vitamin C, resulting in reduced amounts of this vitamin in the blood (73).

Table 8.SNPs related to Vitamin C and their effects.

Vitamin C					
RsID	**Gene**	**Polymorphism function**	**Alleles**	**wt/mt**	**Reference**
rs33972313	SLC23A1	9%-11% lower plasma vitamin C concentrations	T/T	mt/mt	(73,74)
		Lower plasma vitamin C	C/T	wt/mt	
		Typical	C/C	wt/wt	
rs6053005	SLC23A2	24% higher (on average) plasma vitamin C concentrations	T/T	mt/mt	(75,76)
		Typical vitamin C levels	C/T	wt/mt	
		Typical vitamin C levels	C/C	wt/wt	

Variant rs6053005; Gene: SLC23A2

In comparison to people with the C/T or C/C genotypes, those with the T/T genotype for the rs6053005 variant of the SLC23A2 gene typically have greater plasma vitamin C concentrations. This shows that this genetic variation may improve vitamin C efficiency or uptake, resulting in higher amounts of this vitamin in the blood (74).

Conclusion

The population-wide prevention and treatment of vitamin deficiency has been recognized as a key public health goal. Every person has a unique nutritional blueprint stored in their DNA. The scientific field of nutrigenomics studies the relationships between genes and nutrients, enabling the creation of individualized nutrition strategies to promote health and fend off disease. To better understand the interplay between genes and nutrients and to plan tailored weight loss, nutrigenetic testing may soon become a key approach.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review

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Nutrigenomics: SNPs correlated to physical activity, response to chiropractic treatment, mood and sleep

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Abstract

Nutrigenomics, a rapidly evolving field that bridges genetics and nutrition, explores the intricate interactions between an individual's genetic makeup and how they respond to nutrients. At its core, this discipline focuses on investigating Single Nucleotide Polymorphisms (SNPs), the most common genetic variations, which significantly influence a person's physiological status, mood regulation, and sleep patterns, thus playing a pivotal role in a wide range of health outcomes. Through decoding their functional implications, researchers are able to uncover genetic factors that impact physical fitness, pain perception, and susceptibility to mood disorders and sleep disruptions. The integration of nutrigenomics into healthcare holds the promise of transformative interventions that cater to individual well-being. Notable studies shed light on the connection between SNPs and personalized responses to exercise, as well as vulnerability to mood disorders and sleep disturbances. Understanding the intricate interplay between genetics and nutrition informs targeted dietary approaches, molding individual health trajectories. As research advances, the convergence of genetics and nourishment is on the brink of reshaping healthcare, ushering in an era of personalized health management that enhances overall life quality. Nutrigenomics charts a path toward tailored nutritional strategies, fundamentally reshaping our approach to health preservation and preventive measures. ***Clin Ter 2023; 174 Suppl. 2 (6):183-192 doi: 10.7417/CT.2023.2486***

Key words: *Nutrigenomics, physical activity, chiropractic treatment, mood, sleep, SNPs, polymorphism*

Introduction

The discipline of nutrigenomics, at the intersection of genetics and nutrition, stands as a promising frontier, poised to uncover the intricate connection between an individual's genetic makeup and their response to various nutrients (1). This multidisciplinary field holds the potential to reshape how we approach health maintenance through personalized nutrition plans and strategies (2). Central to this realm is the intriguing exploration of Single Nucleotide Polymorphisms (SNPs), genetic variations that wield significant influence over a person's physiological state, encompassing a broad spectrum of facets such as physical performance, emotional demeanor, and sleep patterns (3-5).

The distinct genetic composition intrinsic to each person plays a pivotal role in shaping their unique interaction with nutrients and the environment (6). This interplay between genetics and nutrition forms the basis for tailoring dietary interventions that align with individual requirements and enhance overall health outcomes (7). Researchers and medical practitioners delve into this nexus to unravel how genetic predispositions influence responses to different dietary components (8).

As already mentioned, the bedrock of nutrigenomics lies in the scrutiny of SNPs, the most common form of genetic variation within the human genome (9). These variations involve minute changes in a single nucleotide at specific points in the DNA sequence (10). While some SNPs may have inconspicuous effects, others exert profound impacts on health and susceptibility to diseases (11). This genetic diversity underpins the intriguing variations observed in how individuals react to various dietary elements, serving as the cornerstone for personalized nutrition strategies (12).

Nutrigenomics delves into genetic influences on physical activity and its impact on health. Research has unveiled links between genetic variants and activity levels, sleep, and cardio-metabolic health. Specific genes like ACTN3, ACE, and PPARGC1A play roles in muscle strength, power, endurance, and metabolic adaptation to exercise (13-15). Insights from genetic studies offer potential for personalized fitness strategies and improved performance outcomes (16). Various genetic variants have been associated with

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power, endurance, and muscle function, offering a deeper understanding of the genetic basis of exercise-related traits (17). The impact of genetics on mental well-being and sleep patterns is another facet of nutrigenomics worth exploring. Genetic factors play a crucial role in mood disorders, sleep patterns, and sleep disorders, revealing complex interactions and potential therapeutic avenues (18, 19). The interplay between genetics and sleep has led to the identification of genes impacting sleep timing, structure, and homeostasis, while research on mood disorders has transitioned from single genes to polygenic risk factors and gene-environment interactions (20). Furthermore, genetic insights into sleep disturbances among children with autism and the role of clock genes in sleep homeostasis highlight the intricate relationship between genetics and sleep (21). Genome-wide association studies have contributed to the understanding of genetic links to sleep disorders and mood conditions, offering potential avenues for tailored interventions and improved health outcomes (22).

In this review article, the realm of nutrigenomics reveals an unfolding comprehension of the dynamic interplay between an individual's genetic blueprint and their response to nutrients, as genetics and nutrition converge. Through the lens of Single Nucleotide Polymorphisms (SNPs), this multidisciplinary field presents the prospect of personalized health management, fundamentally reshaping our approach to well-being. Genetic revelations encompassing physical activity, sleep patterns, and mood disorders provide alluring insights into the intricate matrix of interactions that mold human health.

SNPs and Physiological Status

SNPs are variations in a single nucleotide at specific positions in the DNA sequence (23) that can lead to divergent physiological responses to nutrients and environmental stimuli (24). Notably, some SNPs have the potential to exert substantial effects on an individual's physiological status (25). For instance, research has elucidated the influence of certain SNPs on the metabolism of nutrients, such as vitamin absorption, lipid metabolism, and glucose regulation (26). In this context, SCARB1 gene is vital in the reverse cholesterol transport process and lipid metabolism. Research indicates its potential influence on plasma lipid levels and response to interventions. In the GOLDN study, the SCARB1_G2S variant was linked to greater responsiveness to fenofibrate in reducing triglycerides, suggesting its value in predicting lipid level variations and treatment outcomes (27). Another study showed that TLR diversity is positively linked to physiological condition, particularly affecting hemoglobin, albumin, and triglyceride levels, suggesting associations between TLR variation and health indicators in wild avian populations (3). In addition, Yvert et al. assessed a polygenic profile linked to endurance performance using 21 genetic polymorphisms in Japanese endurance runners and controls. The results suggest that while the analyzed genotype score did not significantly influence endurance athlete status, there were some marginal trends indicating potentially higher frequencies of specific genotypes in international athletes (28).

Genetic traits like resistance to stress and physical capacity are crucial for athletes. Examining the COMT gene's rs4680 polymorphism, this study found that athletes with the Met allele had better psychological stability and specific gender-related differences in psychophysiological traits (29). In this context, calpain-calpastatin system is vital for skeletal muscle growth. Research aimed to identify calpastatin gene polymorphisms in sheep and their connection to growth traits; although no significant genotype-trait relationships were found, certain alleles/genotypes showed potential for growth rate preferences (30).

Nutrigenomics: Decoding the Relationship Between Genetics and Physical Activity

At the core of nutrigenomics lies the captivating exploration of polymorphisms and their possible effects on physical activity (31, 32). These genetic variations can exert a profound influence on how the human body metabolizes nutrients and responds to exercise stimuli (33). By unraveling the genetic code underlying nutritional interactions, nutrigenomics opens up new avenues for developing personalized fitness regimens, optimizing training outcomes, and enhancing physical performance (34).

Physical activity, a crucial determinant of health, displays considerable variability among individuals, in part due to genetic factors (35). Qi et al. conducted groundbreaking research using wrist accelerometry data from 88,411 UK Biobank participants, identifying 5 new genetic loci linked to physical activity, sleep duration, and chronotype. Associations extended to activity patterns, sleep, and blood and immune system involvement, with secondary effects on the digestive and endocrine systems (36). Foraita et al. explored the genetic influence on aerobic fitness (AF) measured by maximal oxygen uptake (VO2max), observing a significant familial aggregation of AF and estimating its heritability at 40%. Above-average AF was associated with reduced overweight/obesity risk (37). Angelen et al. investigated the impact of physical activity (PA) and sitting time on cardiometabolic diseases, discovering that low PA and high sitting time significantly increased the risk of cardiovascular disease and metabolic syndrome (38).

Numerous studies highlight the diverse health benefits of regular exercise, which include decreased risk of chronic diseases and enhanced mental health and cognitive function (39). Bailey et al. demonstrated that combining high calcium intake with childhood exercise boosts bone mass accrual and density, especially during the pre-pubertal period (40). Tou et al. examined the complex interplay between activity and body weight regulation, revealing the multifactorial nature of the determinants of physical activity and their implications for weight control (41). Liprinzi et al. revealed an association between physical activity and hearing sensitivity in diabetic U.S. adults, showing that those with hearing loss engaged in less moderate-to-vigorous physical activity (42). Maffulli et al. delved into single-nucleotide polymorphisms (SNPs) in the NDUFV2 gene's connection to lumbar disc degeneration (LDD), finding SNP rs145497186 significantly associated with LDD and chronic low back pain (43).

The Impact of Genetics on Physical Activity: Insights from SNPs

Research has uncovered specific SNPs associated with physical activity and training outcomes. Notably, the ACTN3 gene, with its rs1815739 SNP, has been linked to variations in muscle strength and power performance (44). Individuals with specific genotypes of this SNP may possess a genetic advantage in activities that require explosive power, such as sprinting and powerlifting (45). Similarly, the ACE gene, with its rs4343 SNP, has been associated with an individual's response to endurance training. This SNP influences the enzyme angiotensin-converting enzyme (ACE), which plays a role in cardiovascular function (15). Different ACE genotypes can impact an individual's aerobic capacity and adaptability to sustained physical activity, highlighting the role of genetics in shaping endurance performance. Moreover, the PPARGC1A gene, with its rs8192678 SNP, has implications in metabolic adaptation to exercise. PPARGC1A is a key regulator of mitochondrial biogenesis and oxidative metabolism, influencing an individual's ability to burn energy efficiently during physical activity (14). Genetic variations in this gene can impact an individual's endurance and overall exercise performance.

Table 1 shows some of SNPs involved in physical activity and response to chiropractic treatment.

Exploring the interplay between the ciliary neurotrophic factor (CNTF) 1357 G --> A polymorphism and muscle strength response to upper arm resistance training, Walsh et al. discovered that women with the CNTF GG genotype displayed superior gains in following isometric and dynamic strength trainings (47). Analyzing genetic polymorphisms linked to favorable muscle traits in Italian athletes, Persi et al. identified significant imbalances in ACTN3 R577X and CNTF IVS1-6G>A polymorphisms among athletes compared to controls. The ACTN3 577X/X polymorphism was associated with athlete anaerobic thresholds, hinting at implications for sport performance, training, and neuromuscular disease (13). Genetic variants contributing to elite athlete status were reviewed by Naureen et al., emphasizing that genetic interactions alone do not ensure championship success, due to epigenetic factors and the environment. Genetic testing for sport performance-related polymorphisms was noted to aid in talent identification and training potential assessment (48). Investigating the connection between polymorphisms in the nuclear respiratory factor (NRF2) gene and endurance capacity, He et al. found that certain NRF2 SNPs were associated with variations in endurance capacity and response to training in young Chinese men (80).

Eynon et al. delved into the NRF2 gene variants (rs12594956 and rs8031031) among endurance athletes and sprinters, discovering that specific NRF2 genotypes and alleles were overrepresented in endurance athletes, hinting at their potential role in enhanced endurance performance (50). PPARα G/C polymorphism (rs4253778) was studied among endurance-oriented athletes, power/endurance-oriented athletes, and non-athletes, revealing no significant genotype or allele frequency differences, indicating that the PPARα gene polymorphism might not be a distinct marker for endurance and mixed sport disciplines (51,81). Examining the impact of the PPARA intron 7 G/C polymorphism (rs4253778) on anaerobic power output in elite Czech ice hockey players, Petr et al. found that C allele carriers exhibited higher anaerobic power during the Wingate Test, suggesting a metabolic advantage toward anaerobic metabolism (82). A meta-analysis by Ahmetov et al. demonstrated that the PPARGC1A Gly482Ser polymorphism was significantly associated with sports performance, particularly in power sports and among Caucasian individuals (54). Investigating the influence of PPARGC1A rs8192678 (Gly482Ser) polymorphism on muscle fitness in Chinese schoolchildren, another study unveiled potential associations between this genetic variant and muscle fiber types in girls (55). A study examining the EPAS1 gene's influence on athletic performance found certain genotypes of rs1867785 and rs11689011 underrepresented in sprint/power athletes, suggesting predictive value for sprint/power athletic success (58). Similarly, in elite endurance athletes, EPAS1 gene variations were linked to differences in aerobic and anaerobic metabolism, influencing maximum sustainable metabolic power (57). Investigating the role of genetic variations in AMPD1, CPT2, and PYGM genes in Chronic Fatigue Syndrome (CFS), a study found no major genetic variations associated with CFS in these genes (59). Analyzing the C34T mutation in the AMPD1 gene among top-level Caucasian male endurance athletes and non-athletes, the frequency of the mutant T allele was lower in elite endurance athletes (60). A study on muscle AMPD deficiency in mice revealed no significant impact on muscle performance during different exercise protocols (61). Understanding the cardioprotective effect of the AMPD1 gene variant associated with improved survival in heart failure patients, the study highlighted the metabolic-chronotropic response during exercise as a critical factor (62). Investigating the influence of adenosine in endotoxemia-induced injury, the study found that the AMPD1 variant did not significantly affect inflammation-induced injury during human experimental endotoxemia (63). Exploring the AMPD1 C34T genetic polymorphism in Lithuanian athletes, the study found that the CC genotype was prevalent in sprint/power-oriented athletes and linked to higher short-term explosive muscle power (64).

The influence of genetic variants on exercise reinforcement, tolerance, and moderate-to-vigorous physical activity was studied, revealing associations between certain genotypes and exercise behaviors (65). In a similar way, the association of a DRD2 gene variant with physical activity levels was found to be specific to gender and ethnicity (66). Genetic influences on physical activity were explored further, uncovering associations with genes related to sensation-seeking behaviors (67). Investigating the impact of the AGT gene M235T polymorphism on athletic status and performance level, a study indicated that the CC genotype was overrepresented in power athletes (68); additionally, the very same polymorphism was associated with power-related improvements following aerobic dance training (69). A meta-analysis revealed significant associations between power athlete status and genetic polymorphisms in various genes (70). The AGTR2 rs11091046 polymorphism was found to be associated with sprint/power athlete status in men from Japanese and East European backgrounds (71). The use of ACTN3 R577X genetic polymorphism for personalized training guidelines was explored, linking the gene

Table 1. SNPs Associated with Physical Activity and Response to Chiropractic Treatment.

RsID	Gene	Function	Alleles	wt/mt	References
rs1800169	CNTF	Better response to chiropractic treatment	G/G	mt/mt	(46)
		Typical	G/A	wt/mt	(47)
		Typical	A/A	wt/wt	(13)
rs7181866	GABPB1 (NRF2)	Likely worse in endurance sports	G/G	mt/mt	(48, 49)
		Intermediate phenotype	G/A	wt/mt	(49)
		Likely better in endurance sports and better aerobic capacity	A/A	wt/wt	(50)
rs4253778	PPARA	Likely better in endurance sports and better aerobic capacity	GG	mt/mt	(48)
		Intermediate phenotype	GC	wt/mt	(51)
		Likely better in power sports and lower aerobic capacity	C/C	wt/wt	(52)
rs8192678	PPARGC1A	Likely worse in endurance sports; lower mitochondrial biogenesis and lower increase in insulin sensitivity on aerobic training; likely lower VO2max and higher levels of lactate in blood	A/A	mt/mt	(48, 53)
		Intermediate phenotype	A/G	wt/mt	(54)
		Likely better in endurance sports; higher mitochondrial biogenesis and higher increase in insulin sensitivity on aerobic training; likely normal VO2max and lower levels of lactate in blood	G/G	wt/wt	(55)
rs1867785	EPAS1	Variant rare in the sprint/power athletes	A/A	mt/mt	(48, 56)
		Intermediate phenotype	A/G	wt/mt	(57)
		Typical	G/G	wt/wt	(58)
rs17602729	AMPD1	Loss of enzyme function. May experience muscle soreness in exercise. Possible benefit on cardiovascular function	A/A	mt/mt	(59-62)
		Reduced enzyme function. May experience muscle soreness in exercise. Possible benefit on cardiovascular function	A/G	wt/mt	(63)
		Typical	G/G	wt/wt	(64)
rs6454672	CNR1	Likely to tolerate more high-intensity training	T/T	mt/mt	(65)
		Typical	C/T	wt/mt	(66)
		Typical	C/C	wt/wt	(67)
rs699	AGT	Risk of high blood pressure. Likely to be better in power sports	G/G	mt/mt	(68, 69)
		Slightly higher risk of high blood pressure. Likely to be better in power sports	A/G	wt/mt	(70)
		Typical	A/A	wt/wt	(71)
rs1815739	ACTN3	Functioning protein. More fast, type II muscle fiber. Optimal for elite power athletes	C/C	mt/mt	(48, 72)
		Functioning protein. Optimal for elite power athletes	C/T	wt/mt	(44)
		Non-functioning protein. More likely to be an endurance athlete than power athlete	T/T	wt/wt	(73)
rs1799722	BDKRB2	Typical	C/C	mt/mt	(48)
		Probably better endurance performance, than power performance	C/T	wt/mt	(74)
		Probably better endurance performance, than power performance	T/T	wt/wt	(75)
rs1805086	MSTN	Greater muscle mass	C/C	mt/mt	(46, 76, 77)
		Greater muscle mass	C/T	wt/mt	(76)
		Typical muscle mass, better jumping ability	T/T	wt/wt	(77)
rs2010963	VEGFA	Higher protein levels. Higher improvements in VO2max seen with aerobic training	C/C	mt/mt	(78)
		Higher protein levels. Higher improvements in VO2max seen with aerobic training	C/G	wt/mt	(79)
		Lower protein levels. Lower improvements in VO2max seen with aerobic training	G/G	wt/wt	(79)

variant to muscle phenotypes (72). Investigating the ACTN3 gene's impact on athletic performance and muscle function, the study found that overexpression of α-actinin-3 altered muscle metabolism and fatiguability, challenging previous assumptions (73). The influence of genetic polymorphisms on endurance performance was examined, revealing specific gene variants (BDKRB2 and ADRB2) with potential implications for endurance performance among habitual runners (74). The associations between gene variants in muscle afferents and exercise pressor responses were explored, with specific variants in TRPV1 and BDKRB2 receptors showing significance (75). Genetic polymorphisms (MSTN 2379 A > G and FST -5003 A > T) were examined in relation to muscle size and strength responses to resistance training across various ethnic groups (76). In the context of the MSTN K153R polymorphism, a study indicated that the KR genotype was linked to lower performance in vertical jumps, suggesting its potential influence on muscle power during contractions (77).

Nutrigenomics: Decoding the Relationship Between Genetics and Pain Perception

Chiropractic is an alternative therapy based on the body's self-healing ability and the relationship between body structure and health (46). It is widely used for chronic pain treatment, but the exact molecular mechanisms are not fully understood. Animal studies suggest that chiropractic impacts neuroplasticity through neurotrophin modulation. No published research has explored the interaction between neurotrophin gene polymorphisms and chiropractic treatment. The study identified potential genes and polymorphisms correlated with a better response to chiropractic therapy. However, more association studies are needed to confirm these findings.

The study explored associations between NTRK1 gene SNPs and pain perception in a Han Chinese population (4). Nine tag-SNPs of NTRK1 showed significant associations with pressure pain thresholds, leading to hyper- or hyposensitivity. Specifically, four tag-SNPs (rs1800880, rs6334, rs2644604, and rs943552) were highly associated with lower mechanical pain sensitivity to sharp pressure pain, and individuals with the haplotype CTCC exhibited hyposensitivity to sharp pressure pain compared to other haplotypes.

A research investigation centered on the molecular mechanisms of chiropractic therapy employed "chiropractic," "neuroplasticity," and "neurotrophin gene polymorphism" as focal points. The analysis revealed specific genes and functional polymorphisms that might be linked to a more favorable response to chiropractic therapy (46). Another study delved into the connections between NTRK1 gene SNPs and pain perception in Han Chinese females. Nine tag-SNPs of NTRK1 displayed significant associations with pressure pain thresholds, manifesting as either hyper- or hyposensitivity. Notably, certain tag-SNPs (rs1800880, rs6334, rs2644604, and rs943552) were markedly related to reduced mechanical pain sensitivity, with the CTCC haplotype particularly linked to hyposensitivity (4).

Genetics, Mood, and Sleep

The impact of genetic factors on mood and sleep patterns is an area of increasing interest (5). Specific genetic variants have been associated with an increased risk of mood disorders, such as depression and anxiety, while others may influence an individual's sleep quality and circadian rhythms (18). In this context, a meta-analysis of large-scale studies involving over 807,000 individuals revealed significant associations between 102 variants, 269 genes, and 15 gene-sets with depression, shedding light on its genetic complexity and potential treatment avenues (19). Children on the autism spectrum often experience sleep problems, which can worsen emotional and behavioral challenges. This study aimed to identify genetic variants associated with sleep disturbance and melatonin levels in autistic children, revealing potential distinct biological mechanisms underlying these issues (20). Circadian and sleep-homeostatic processes both influence sleep timing and structure. Recent evidence suggests that clock genes, traditionally associated with circadian rhythms, also play a role in sleep homeostasis, impacting sleep duration, structure, and EEG delta power across species (21). Researchers observed increased expression of circadian clock-genes in the cerebral cortex during sleep deprivation (SD), with the magnitude of increase corresponding to the extent of sleep rebound after SD in different mouse strains. Specifically, elevated per2 expression persisted in mice with limited sleep rebound, suggesting its potential role in negatively influencing recovery sleep (87). Rare variants in specific genes have been linked to Mendelian sleep conditions, but their effects in the general population are unclear. This study examined these variants in large cohorts and found that they are not highly penetrant for extreme sleep

Table 2. SNPs Associated with Pain Perception

RsID	Gene	Function	Alleles	wt/mt	References
rs6746030	SCN9A	Increased perception of pain	A /A	mt/mt	(46)
		Somewhat increased perception of pain	A /G	wt/mt	(83)
		Typical	G /G	wt/wt	(84)
rs6334	NTRK1	Increased pain perception during acupuncture	A/A	mt/mt	(4, 46)
		Somewhat increased pain perception during acupuncture	A/G	wt/mt	(85)
		Typical	G/G	wt/wt	(86)

or circadian phenotypes, suggesting that their impact may differ in a broader population context (88).

Mood disorders have a strong genetic component, and research in this field has evolved from focusing on single genes to polygenic risk factors and gene-environment interactions. This scientometric analysis highlights shifts in research trends, from monogenic studies to genome-wide association studies and the exploration of genetic overlaps with other psychiatric conditions, as well as the increasing importance of gene-environment interactions in understanding mood disorder risk (22). As the number of previous depressive episodes increases, the link between stressful life events and the onset of major depression weakens. This study investigated the impact of genetic risk factors on this phenomenon and found that individuals at high genetic risk for depression tend to experience depressive episodes without major environmental stressors, suggesting a "pre-kindling" effect rather than an accelerated kindling process (89). Genetic variants associated with cardiovascular and metabolic diseases, as well as mood disorders, have been identified through meta-analyses and candidate gene studies. This article reviews and analyzes shared genes, linked to both cardiometabolic diseases and mood disorders, identifying 24 potential pleiotropic genes and revealing significant shared pathways (90). Sleep is vital, yet its functions remain largely unknown, and disruptions can lead to health issues. Genetic factors influence sleep variation and disorders. Genome-wide association studies have identified genetic variants associated with sleep disorders, like insomnia and sleep apnea, offering valuable insights for prevention and treatment (91).These findings underscore the importance of considering genetic factors in tailoring therapeutic interventions for mood-related conditions (92). Investigating the connection between the neurotrophin Nerve Growth Factor (NGF) and pain perception, another study disclosed that mutations in NGF-related genes led to hereditary pain insensitivity disorders (HSAN IV and HSAN V), along with diverse cognitive neurological effects. Specifically, the R100W mutation in mature NGF reduced pain-inducing activity in mice, offering insights into HSAN V clinical manifestations and the role of NGF receptors and signaling cascades in pain sensitization (93).

Table 3 shows some of SNPs involved in mood and sleep activities.

Discussion

The study of SNPs correlated to physical activity, pain perception, mood, and sleep patterns has significant implications for personalized nutrition and healthcare interventions (5, 36, 93). Understanding an individual's genetic predisposition to these various aspects of health can inform targeted interventions and improve overall well-being (100).

The investigation of SNPs correlated to physical activity holds immense significance, offering valuable insights into optimizing exercise regimens and promoting overall well-being (101). By understanding an individual's genetic predisposition to various aspects of physical performance, nutrigenomics empowers healthcare practitioners to design exercise programs that capitalize on genetic strengths and address weaknesses, thereby optimizing training outcomes and enhancing physical performance. Certain genes have emerged as key players in the context of physical activity and chiropractic response, warranting further investigation. Genes such as ACTN3, ACE, PPARGC1A, OPRM1, COMT, and SCN9A play critical roles in shaping an individual's physical performance and pain perception (102, 103). Delving deeper into the interactions between these genes and their associated SNPs will unlock new possibilities for targeted interventions, personalized healthcare, and improved treatment outcomes.

Likewise, insights into the genetic influences on mood and sleep patterns offer opportunities for personalized mental health management (18). Indeed, understanding the genetic influences on mood and sleep patterns presents exciting prospects for personalized mental health management (104), in order to help millions of people worldwide managing conditions like depression, anxiety, and sleep disorders (105). However, the susceptibility to these conditions and response to treatment can vary widely among individuals (106). Nutrigenomics offers a novel perspective, by investigating how genetic factors contribute to mood regulation and sleep patterns, potentially revolutionizing mental health care (107).

Table 3. SNPs Involved in Mood and Sleep and Response to Chiropractic Treatment

RsID		Gene	Function	Alleles	wt/mt	References
rs334558		GSK3B	Increased risk for severe insomnia	G/G	mt/mt	(94)
		GSK3B	Increased risk for severe insomnia	A/G	wt/mt	(95)
		GSK3B	Typical	A/A	wt/wt	(96)
rs73598374		ADA	Typical	C/C	wt/wt	(97)
		ADA	Reduced clearance of adenosine. May lead to more deep sleep, but sleepiness when waking up	C/T	wt/mt	(98)
		ADA	Reduced clearance of adenosine. May lead to more deep sleep, but sleepiness when waking up	T/T	mt/mt	(99)
rs6330	NGF		More anxiety in females, less anxiety males	C/C	mt/mt	(92)
			Typical	T/C	wt/mt	(92)
			More anxiety in males, less anxiety females	T/T	wt/wt	(93)

The identification of specific SNPs associated with mood disorders has been a focus of extensive research. In this context, Fraizer et al. found associations between specific genetic variants (ANK3, BDNF, CACNA1C, DGKH) and mood disorders, cognition, and brain regions involved in affect regulation. Among these variants, CACNA1C carriers showed larger fronto-limbic brain volumes, increased IQ, and potential associations with mood disorder-related systems, suggesting a potential marker for neuropsychiatric risk (108). Similarly, research into anxiety-related SNPs, such as those in the COMT gene, sheds light on the genetic basis of anxiety susceptibility (109). By understanding the genetic variants associated with anxiety risk, mental health practitioners can better tailor treatment plans, including psychotherapy and pharmacotherapy, to suit individual needs.

Moreover, sleep is a vital component of overall well-being, and genetic factors can significantly impact an individual's sleep patterns (109). The study by Riestra et al. (2017) on SNPs in the CLOCK gene revealed how genetic variations could influence sleep duration and quality (110). This finding opens up opportunities for personalized sleep management strategies, such as chronotherapy or personalized sleep hygiene recommendations, to address sleep disturbances effectively.

Integrating nutrigenomics into mental health management can lead to more precise and targeted interventions (111). For instance, identifying an individual's genetic susceptibility to depression may inform treatment decisions, guiding the selection of medications that are more likely to be effective for that specific genetic profile (112). Additionally, personalized lifestyle modifications, including dietary and exercise recommendations tailored to the individual's genetic makeup, can complement conventional therapies and improve treatment outcomes (113, 114).

However, while nutrigenomics offers great promise, several challenges must be addressed for its successful integration into mental health care (115). Ethical considerations related to genetic testing and privacy must be carefully navigated, to ensure that individuals' rights and autonomy are respected (116). Genetic counseling should be made readily available, to help individuals understand the implications of their genetic information and make informed decisions about their mental health management (117).

Furthermore, the complexities of gene-environment interactions should be thoroughly investigated to comprehensively understand the interplay between genetics, lifestyle, and environmental factors in mental health (118). This knowledge will enable a holistic approach to personalized mental health care that considers the dynamic interactions between genes and the environment.

Conclusions

Nutrigenomics, with its focus on understanding the interplay between genetics and nutrition, represents a pioneering approach to personalized medicine and healthcare. The investigation of Single Nucleotide Polymorphisms (SNPs) and their correlations to physical activity, pain perception, mood, and sleep patterns provides invaluable insights into individual health and well-being. By leveraging genetic information, personalized nutrition plans and exercise regimens can be tailored to maximize health benefits and optimize fitness outcomes. Understanding an individual's genetic predisposition to various health aspects can guide targeted interventions, promoting physical activity, pain perception, and sleep and mood patterns. In the realm of mental health, nutrigenomics presents exciting opportunities for personalized mental health management. Identifying specific genetic markers associated with physical activity, pain perception, mood disorders, and sleep patterns can facilitate early detection and intervention, leading to more effective treatment approaches. As nutrigenomics continues to evolve, further research and advancements are needed to fully unlock its potential in transforming healthcare. Embracing the possibilities of nutrigenomics could move us towards a future of precision medicine, where healthcare is tailored to each individual's unique genetic composition, ultimately enhancing overall health and quality of life.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review

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Nutrigenomics: SNPs correlated to minerals' deficiencies

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Abstract

Nutrigenetics and nutrigenomics are two interrelated fields that explore the influence of genetic diversity on nutrient responses and function. While nutrigenetics investigates the effects of hereditary genetic variations on micronutrient metabolism, nutrigenomics examines the intricate relationship between diet and the genome, studying how genetic variants impact nutrient intake and gene expression. These disciplines offer valuable insights into predicting and managing chronic diseases through personalized nutritional approaches.

Nutrigenomics employs cutting-edge genomics technologies to study nutrient-genome interactions. Key principles involve genetic variability among ethnic groups, affecting nutrient bioavailability and metabolism, and the influence of dietary choices based on cultural, geographic, and socioeconomic factors. Polymorphisms, particularly single-nucleotide polymorphisms (SNPs), significantly influence gene activity and are associated with specific phenotypes that are related to micronutrient deficiencies.

Minerals are inorganic elements, vital for various physiological functions. Understanding the SNPs associated with mineral deficiencies is crucial for assessing disease risk and developing personalized treatment plans. This knowledge can inform public health interventions, targeted screening programs, educational campaigns, and fortified food products to address deficiencies effectively. Nutrigenomics research has the potential to revolutionize clinical and nutritional practices, providing personalized recommendations, enhancing illness risk assessment, and advancing public health initiatives. Despite the need for further research, harnessing nutrigenomics' potential can lead to more focused and efficient methods for preventing and treating mineral deficiencies. ***Clin Ter 2023; 174 Suppl. 2 (6):193-199 doi: 10.7417/CT.2023.2487***

Key words: *Nutrients, SNPs, nutrigenomics, biomarkers, genes, minerals, calcium, potassium, iron, magnesium, selenium*

Introduction

The field of nutrigenetics studies the impact of genetic diversity on nutrient responses and functions. Although closely linked, nutrigenomics and nutrigenetics are not the same thing. Nutrigenetics investigates the effects of hereditary genetic variations on the uptake and metabolism of micronutrients, whereas nutrigenomics studies the connection between "diet and the genome with reference to nutritional effects on the metabolic, proteomic, transcriptional, and translational changes as well as dietary variation due to an individual's genetic background (1). Numerous chronic diseases can be predicted using nutrigenetic research; moreover, by using individualized nutritional management, these conditions may be avoided or treated more effectively. The majority of nutrigenetic experiments investigate how various polymorphisms influence alterations in eating patterns (2). Since people who carry certain polymorphisms in the apolipoprotein E gene are at a higher risk of myocardial infarction (MI), diets that are customized for such individuals should reduce the amount of saturated fats consumed in comparison to the conventional dietary guidance (3).

Through major metabolic capacity changes, brought about by diet over the history of human development, modern illnesses have become more prevalent. From an evolutionary standpoint, nutrition, like other environmental factors, is a limiting factor that places selective pressure on a population (4). A population's genotypes vary in their nutrient requirements. However, until these requirements (e.g., the need for additional calories from carbs and dietary fat) are addressed, the gene that transmits the high dietary requirement will continue to persist in the population. For example, this might apply to genes linked to diabetes and obesity (5).

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To characterize the relationship between nutrients and genes, the term "Nutrigenomics" was coined. Physiology, biochemistry, metabolomics, proteomics, transcriptomics, and bioinformatics are all connected to genetics through nutrigenomics (6). In this developing discipline, cutting-edge genomics methods are used to examine how nutrients affect the genome and gene expression, as well as how genetic variations affect nutrient consumption: for example, numerous genetic variations have been shown to affect the way proteins are built and function. The study of how dietary factors affect people in different ways, according to their genetic makeup, is known as nutrigenetics, and it is a sub-field of dietary genome research. Nutrigenomics investigates how dietary factors interact with the genome to regulate modifications to proteins and other metabolic functions (7).

Nutrigenomics

The purpose of the developing field of nutrigenomics, which employs the most advanced genomics technologies available, is to examine the effects of vitamins on the genome and gene expression, as well as the effects of genetic variants on the intake of nutrients. Ethnic groups' genetic variability may have an impact on the bio-availability of nutrients and how they are metabolized (8), since the choice of food and its accessibility are largely based on cultural, geographic, and socioeconomic considerations. Because nutrition affects DNA sequences or even causes chromosomal instability, which results in abnormal gene dosage and undesired phenotypes, it also threatens the stability of the genome. As a result, the field of nutritional research known as "nutrigenomics" focuses on the application of molecular tools to the process of examining, assessing, and understanding the physiological reactions of certain populations or people to various diets (9).

The ability of particular nutrients to connect with transcription factors is necessary for these gene-nutrient interactions, which in turn control the attraction of RNA polymerase to promoter regions of genes and the amounts of transcripts that are generated as a result. For instance, research on fatty acids and vitamins A and D has shown that these nutrients effectively engage nuclear receptors and trigger gene transcription (10). Additionally, compounds like genistein (from soy) and resveratrol (from wine) indirectly affect numerous molecular signaling pathways through "nuclear factor kappa B," which ultimately results in the stimulation and control of important disease-related proteins (11).

Polymorphisms and their possible effects

In the context of genomics, the term "polymorphism" refers to the occurrence of two or more alternative variants of a certain DNA sequence in various individuals or populations. The most frequent kind of polymorphisms (also known as a single-nucleotide polymorphisms, or SNPs) involves variation at a single nucleotide; other polymorphisms can involve longer sections of DNA and thus be substantially larger (12). In the last two decades, nutrigenomics research has gathered enough information to distinguish between the phenotypes of SNPs in populations with and without micronutrient deficiencies. SNPs are the most frequent type of variation in human DNA (13), and can significantly affect gene activity (14).

Minerals and their role

Minerals are inorganic materials, necessary for the healthy operation of the human body and other living things. They are essential for many physiological functions and trace levels of them are needed for overall health and wellbeing (15). Major minerals (or "macro minerals") and trace minerals ("micro minerals") are two categories used to describe essential minerals: the first are needed in larger quantities, while the latter are needed in smaller ones. All of the necessary minerals should be present in a balanced diet (16).

The human body uses minerals in a variety of ways. For example, calcium is essential for the growth and upkeep of healthy bones and teeth, the immune system and energy generation both depend on iron, and magnesium is needed in countless metabolic processes. Potassium is an electrolyte that helps control fluid balance, neuron activity, and muscle contractions (16); while zinc participates in a variety of enzymatic processes and is essential for cell growth, wound healing, and immunological function. Iodine is required for the synthesis of thyroid hormones—which control growth, development, and metabolism—and selenium, thanks to its antioxidant properties, aids in preventing cell deterioration (17). The production of red blood cells, the development of connective tissue, and the functioning of the neurological and immunological systems all depend on copper. Each mineral serves a distinct purpose, so it is crucial to get enough of them through a balanced diet to preserve optimum health (18).

Why studying SNPs correlated to minerals deficiencies can be important

SNPs, which are variations in our DNA, can impact how our bodies absorb, transport, and use nutrients. Researchers can learn more about a person's genetic propensity for mineral deficiencies by analyzing the SNPs that are linked to these deficiencies, which are themselves linked to a higher risk of acquiring several illnesses. So, finding SNPs linked to mineral deficiencies can also help in the development of specialized treatment plans (19).

The field of nutrigenomics investigates the interactions between genes and nutrition and how they impact our health. For the purpose of developing public health interventions and policy, research on SNPs associated with mineral deficiencies can yield useful information. It helps in identifying population subgroups whose genetic make-up may make them more vulnerable to particular mineral deficiencies (20). To address and prevent deficiencies in susceptible populations, this knowledge can direct focused screening programs, educational campaigns, and the creation of fortified food products. Studying mineral deficiencies-associated SNPs can have a big impact on nutrigenomics research, individualized nutrition, illness risk assessment, treatment plans, and public

health initiatives (21). Moreover, it advances our knowledge of the genetic influences on mineral metabolism and creates opportunities for more focused and efficient methods to treat and prevent said deficiencies (22).

Calcium

Calcium is a necessary mineral, required for the upkeep of healthy bones and teeth. Additionally, it promotes healthy nerve transmission, blood coagulation, and hormone release.

Being involved in many cellular functions, calcium is important for overall health and wellbeing. Several foods—especially dairy goods like milk, cheese, and yogurt—are excellent providers of calcium. Muscle cramps and spasms, tingling in the fingers and toes, brittle bones, poor tooth health, and an increased risk of dental issues are just a few indications of a calcium deficiency, also known as hypocalcemia (23).

SNPs correlated to calcium deficiency and their effects

A person's chance of being calcium deficient can be affected by genetic factors affecting calcium metabolism. The gene for the vitamin D receptor is one of many SNPs connected to calcium metabolism. This SNP has been associated with variations in bone mineral density and calcium absorption, which may impact a person's vulnerability to calcium deficiency and associated diseases (24).

Dietary supplementation of calcium deficiency

Calcium supplements may be useful in cases of confirmed calcium deficiency or when its intake through food consumption is insufficient. Calcium supplements come in a variety of forms, including calcium citrate and calcium carbonate. To prevent potential adverse effects and guarantee proper absorption, it's crucial to adhere to the dosage and duration recommendations of healthcare professionals when using supplements. Supplements can be mixed with other nutrients, like vitamin D, which helps the body absorb calcium (25).

Table 1 gives details on a specific SNP (rs1800012) in the COL1A1 gene, the gene's function, the polymorphism's role, and the relevant alleles. Type I collagen, a kind of fibrillary collagen present in a variety of connective tissues, including cartilage, is mostly encoded by the COL1A1 gene. Collagen gives bones, tendons, ligaments, and other connective tissues structural support. Lower bone mineral density is linked to the COL1A1 gene variant or SNP (rs1800012).

Iron

Iron is a necessary mineral that is important for many bodily processes, including DNA synthesis, oxygen delivery, body energy production, immune system improvement, and cognitive growth. Both animal and plant-based foods contain iron (26): red meat, chicken, fish, shellfish, legumes, dark leafy greens, nuts, seeds, and grains and cereals are a few examples. Iron deficiency anemia is a disorder that can result from a lack of iron and that causes typical symptoms, such as weakness and exhaustion, shortness of breath, pale skin and nail beds, numbness in the hands and feet, and brittle nails (26).

SNPs correlated to iron deficiency and their effects

SNPs have an effect on iron metabolism and raise the risk of iron dysregulation (Table 2). Hereditary hemochromatosis, a condition marked by increased iron absorption and iron overload in the body, is linked to these mutations. However, it's crucial to remember that nutritional and environmental factors also play major roles in iron deficiency, and hereditary factors are only one component of this condition (27).

Dietary supplementation of iron deficiency

Under the direction of a healthcare practitioner, nutritional supplementation may be suggested in cases of iron deficiency. There are several types of iron supplements, and the selection of the correct one is influenced by many factors, including iron levels, toleration, and personal demands. It is thus crucial to seek medical advice in order to choose the right iron supplements and their dosage and duration. Following the suggested dosage is especially important, because also too much iron might have negative effects on health (28).

Magnesium

Magnesium is a necessary mineral that is vital to many body processes. For example, magnesium plays a role in the synthesis and storage of ATP, in muscle contraction and relaxation, in promoting the healthy functioning of the neural system and assisting in nerve transmission, and in maintaining strong bones by regulating calcium levels. Magnesium can be found in a number of foods, including vegetables with green leaves, seeds grains, fish and spinach. Magnesium deficiency symptoms include muscle cramps, fatigue, weakness, irregular heartbeat and palpitations, nausea, vomiting, and loss of appetite (31).

Table 1. SNPs related to calcium and their effects.

Calcium					
RsID	Gene	Polymorphism function	Alleles	wt/mt	Reference
rs1800012	COL1A1	Lower Bone Mineral Density	A/A	mt/mt	(23-25)
		Lower Bone Mineral Density	A/C	wt/mt	
		Typical	C/C	wt/wt	

Table 2. SNPs related to iron and their effects.

Iron					
RsID	Gene	Polymorphism function	Alleles	wt/mt	Reference
rs17342717	SLC17A1	Higher ferritin.	T/T	mt/mt	(26)
		Higher ferritin.	C/T	wt/mt	
		Typical	C/C	wt/wt	
rs1800562	HFE	High ferritin levels.	A/A	mt/mt	(27)
		Increased ferritin levels.	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs855791	TMPRSS6	Lower ferritin levels.	A/A	mt/mt	(28)
		Lower ferritin levels.	G/A	wt/mt	
		Typical	G/G	wt/wt	
rs3923809	BTBD9	Higher ferritin.	G/G	mt/mt	(29)
		Higher ferritin.	A/G	wt/mt	
		Typical	A/A	wt/wt	
rs7385804	TFR2	Lower serum iron.	C/C	mt/mt	(26,27,30)
		Lower serum iron.	A/C	wt/mt	
		Typical	A/A	wt/wt	
rs3811647	TF	Higher ferritin.	A/A	mt/mt	(23-25)
		Higher ferritin.	A/G	wt/mt	
		Typical	G/G	wt/wt	

SNPs correlated to magnesium deficiency and their effects

SNPs have the potential to affect magnesium metabolism and cause magnesium deficiency. Lower amounts of magnesium in the body may result from polymorphisms that influence magnesium absorption and reabsorption. However, it is crucial to remember that also nutritional and environmental factors, in addition to genetic ones, play a big part in magnesium shortage (32).

Dietary supplementation of magnesium deficiency

Under the direction of a healthcare expert, nutritional supplementation may be suggested in cases of magnesium insufficiency. There are several types of magnesium supplements, including magnesium oxide, magnesium citrate, and magnesium glycinate. The selection of a supplement is influenced by things like absorption, toleration, and individual requirements (33).

The consequences of particular SNPs connected to magnesium are detailed in Table 3.

Selenium

Selenium is a trace mineral, essential for many physiological activities. The human body uses selenium for a variety of purposes, including antioxidant activity, thyroid and immunological functions, reproductive health, DNA synthesis and repair, and cognitive function. Brazil nuts, shellfish, meat and poultry, whole grains, milk and yogurt, eggs, lentils, and other seeds are among the foods that contain selenium. Deficiency signs can include mood swings, hair loss, increased susceptibility to infections, weakened immune system, thyroid malfunction, and muscle weakness and weariness (37).

SNPs correlated to Selenium deficiency and their effects

The metabolism of selenium and its possible effect on deficiency have been linked to SNPs (Table 4). Variations in these genes may have an impact on the expression or activity of selenium-related enzymes, which may have an impact on selenium status and associated health effects. However, the effects of these SNPs may differ between people, and more study is required to properly comprehend their implications (37).

Dietary supplementation of selenium deficiency

If dietary selenium requirements cannot be satisfied by diet alone, or if a person has a confirmed lack of the mineral, taking selenium supplements may be advised. To prevent an excessive intake of selenium, which can be dangerous, a healthcare practitioner should decide on the right quantity and duration of supplementation, based on each patient's needs and lab tests. A medical expert should be consulted before beginning any supplements regimen, since they can offer individualized advice based on certain health issues and factors (38).

Zinc

Zinc is a mineral that is part of numerous enzymatic complexes and is necessary for the proper functioning of many hormones, including insulin, growth hormone, and

Table 3. SNPs related to magnesium and their effects.

Magnesium					
RsID	Gene	Polymorphism function	Alleles	wt/mt	Reference
rs3750425	TRPM6	Lower serum magnesium levels; increased risk of hypomagnesia with proton pump inhibitors.	T/T	mt/mt	(31-33)
		Lower serum magnesium levels; increased risk of hypomagnesia with proton pump inhibitors.	C/T	wt/mt	
		Typical	C/C	wt/wt	
rs12255372	TRPM6	Lower magnesium levels on average; increased risk of hypomagnesia with proton pump inhibitors.	C/C	mt/mt	(31-33)
		Lower magnesium levels on average; increased risk of hypomagnesia with proton pump inhibitors.	C/T	wt/mt	
		Typical	T/T	wt/wt	
rs11191548	CNNM2	Higher levels of 25-hydroxyvitamin D (main circulating form of vitamin D)	C/C	mt/mt	(34-36)
		Higher levels of 25-hydroxyvitamin D (main circulating form of vitamin D)	C/T	wt/mt	
		Typical	T/T	wt/wt	

sex hormones. Zinc is present in various foods: fish and meat, grains, legumes, nuts, and seeds. The Recommended Daily Intake is 11 mg per day for men, while for pregnant and lactating women it is 13 mg per day (39).

SNPs correlated to zinc deficiency and their effects

Several SNPs have been identified that are associated with zinc metabolism and homeostasis, influencing the risk of developing zinc deficiency. These SNPs primarily affect genes involved in zinc transport, absorption, and utilization within the body.

One such SNP is found in the SLC39A8 gene (Table 5), encoding a zinc transporter responsible for facilitating zinc uptake into cells. Variants of this gene have been linked to altered zinc levels and potential zinc deficiency due to impaired transport. Another SNP in the SLC30A8 gene, which encodes a zinc transporter involved in insulin production, has been associated with both reduced zinc levels and an increased risk of type 2 diabetes (40,41).

Zinc deficiency plays a crucial role in various physiological processes, including immune function, growth, wound healing, and cognitive development. Individuals with SNPs related to zinc deficiency may be more susceptible to immune system dysfunction, impaired wound healing, and cognitive deficits. Additionally, zinc deficiency has been associated with increased susceptibility to infections, impaired taste and smell perception, and skin issues (42).

Dietary supplementation of selenium deficiency

Dietary supplementation is a key strategy to address zinc deficiency. Aiming to meet the recommended dietary allowances for zinc can help prevent and alleviate the adverse effects of deficiency. However, in cases where genetic variations predispose individuals to zinc deficiency, targeted supplementation might be beneficial. Supplementing with zinc in cases of genetically influenced deficiency can support immune function, aid wound healing, and promote overall health. Zinc supplements are available in various forms, such as zinc gluconate, zinc sulfate, and zinc citrate. It's important to note that excessive zinc supplementation can lead to adverse effects, including impaired copper absorption and gastrointestinal disturbances (42,43).

Conclusions

Many genetic polymorphisms can influence the levels of minerals in the human organism, modifying the nutritional and health status of the individual. Although further research

Table 4. SNPs related to selenium and their effects.

Selenium					
RsID	Gene	Polymorphism function	Alleles	wt/mt	Reference
rs3877899	SELENOP	Lower serum selenium levels	T/T	mt/mt	(37,38)
		Lower serum selenium levels	C/T	wt/mt	
		Typical	C/C	wt/wt	

Table 5. SNPs related to zinc and their effects.

Zinc					
RsID	Gene	Polymorphism function	Alleles	wt/mt	Reference
Rs13266634	SLC30A8	Lower zinc level, increased glucose levels in blood	C/C	mt/mt	(40,41)
		Lower zinc level, increased glucose levels in blood	C/T	wt/mt	
		Typical	T/T	wt/wt	

is needed to identify possible new SNPs correlated to minerals' absorption, transport, and metabolism, the study of nutrigenomics can be exploited in clinical and nutritional practices to provide tailored suggestions.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review

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Nutrigenomics: SNPs Correlated to Lipid and Carbohydrate Metabolism

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Abstract

Background. Nutrigenomics - the study of the interactions between genetics and nutrition - has emerged as a pivotal field in personalized nutrition. Among various genetic variations, single-nucleotide polymorphisms (SNPs) have been extensively studied for their probable relationship with metabolic traits.

Methods. Throughout this review, we have employed a targeted research approach, carefully handpicking the most representative and relevant articles on the subject. Our methodology involved a systematic review of the scientific literature to ensure a comprehensive and accurate overview of the available sources.

Results. SNPs have demonstrated a significant influence on lipid metabolism, by impacting genes that encode for enzymes involved in lipid synthesis, transport, and storage. Furthermore, they have the ability to affect enzymes in glycolysis and insulin signaling pathways: in a way, they can influence the risk of type 2 diabetes. Thanks to recent advances in genotyping technologies, we now know numerous SNPs linked to lipid and carbohydrate metabolism. The large-scale studies on this topic have unveiled the potential of personalized dietary recommendations based on an individual's genetic makeup. Personalized nutritional interventions hold promise to mitigate the risk of various chronic diseases; however, translating these scientific insights into actionable dietary guidelines is still challenging.

Conclusions. As the field of nutrigenomics continues to evolve, collaborations between geneticists, nutritionists, and healthcare providers are essential to harness the power of genetic information for improving metabolic health. By unraveling the genetic basis of metabolic responses to diet, this field holds the potential to revolutionize how we approach dietary recommendations and preventive healthcare practices. ***Clin Ter 2023; 174 Suppl. 2 (6):200-208 doi: 10.7417/CT.2023.2488***

Key words: *Nutrigenomics, single-nucleotide polymorphisms, SNP, lipid metabolism, carbohydrate metabolism, personalized nutrition, metabolic disorders.*

Introduction

In recent years, the intersection of genetics and nutrition has garnered significant attention within the field of molecular biology and health sciences: in particular, the field of nutrigenomics, which focuses on elucidating the intricate relationship between an individual's genetic makeup and their dietary responses, has been in the limelight (1). To better understand what nutrigenomics encompasses, it is pivotal to comprehend some basic concepts. So far, it is well-established that every human being is unique; however, each person also has a unique nutritional blueprint inside their genes, the expression of which can be influenced by various bioactive food nutrients (2). Furthermore, genomic diversity varies among different ethnic groups, thus affecting nutrients bioavailability and their metabolism. Nutrigenomics usually involves multiple fields, including nutrition, molecular biology, epidemiology, bioinformatics, and genomics. It primarily uncovers how genetic variations influence the body's metabolism, nutrient absorption, and overall health outcomes (3).

The concept that individuals respond differently to the same diet is not new. However, recent breakthroughs in genetic sequencing have enabled researchers to explore the underlying genetic factors responsible for these variations (4). The holistic approach of nutrigenomics bridges the gap between genetics, nutrition, and health, aiming to develop tailored dietary recommendations for optimal well-being. This is particularly relevant in conditions that are influenced by both genetic and nutritional components. Genetic variations have been implicated in the development of various conditions—including gastrointestinal cancers, many gastrointestinal disorders, and inflammatory diseases (5, 6). Imbalances in nutrient levels contribute to issues like the aging process, alcoholism, various types of cancer, cardiovascular diseases (CVDs), hearing impairment, immune system dysfunctions, diabetes, and stroke (7).

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Nutrigenomics and Obesity

Obesity is deemed a core element of metabolic disorders, which usually includes impaired glucose tolerance, hyperinsulinemia, and noninsulin-dependent diabetes mellitus. The susceptibility to developing obesity is dependent upon genetically determined patterns of energy balance regulation (8). In the last ten years, significant advancements have unveiled numerous polymorphic genes responsible for regulating both central and peripheral factors, influencing energy intake and expenditure. Polymorphisms within these genes can impact the regulation of food consumption. These genes encompass taste receptor coding genes as well as various signaling peptides (like insulin, leptin, ghrelin, and cholecystokinin) along with their respective receptors, which collectively play a pivotal role in controlling food intake (9). Hypothalamic neuropeptide Y, agouti-related protein, and various factors within the melanocortin pathway are among the central regulators responsible for polymorphic energy intake (10, 11). Furthermore, significant genetic variations have been identified in the genes governing energy expenditure modulation. These genes include alpha and beta-adrenoceptors, uncoupling proteins, as well as regulators involved in the growth and differentiation of adipocytes (12).

Another aspect of obesity is inflammation. In obese individuals, macrophages infiltrate adipose tissues and stimulate the release of inflammatory molecules, including interleukin-6 (IL-6), interleukin-1β (IL-1β), interleukin-8 (IL-8), and tumor necrosis factor-α (TNF-α), along with inflammatory modulators, like adiponectin and leptin (13, 14). Nutritional components are key factors that can modulate metabolic inflammation in obese individuals (15). Diets rich in sugars and refined grains can increase the expression of pro-inflammatory cytokines. On the other hand, the Mediterranean diet (MedDiet), being rich in monounsaturated fatty acids, can decrease the expression of inflammatory cytokines (16-19); similarly, fruits and vegetables can provide adequate quantities of health-promoting bioactive compounds (20, 21). In a randomized control trial by Camargo et al., the effect of MedDiet on reducing pro-inflammatory gene expression was validated. Their findings showed that MedDiet, enriched with virgin olive oil, lowered the expression of NF-kB p65 gene and elevated IkB α gene expression (22). NF-kB has been linked with elevated levels of proinflammatory cytokines, such as IL-6 and TNF-α (23).

Nutrigenomics and CVDs

Similar to obesity, CVDs are also influenced by a combination of genetic factors and environmental factors (diet). Research has established a strong link between diet composition and the risk of CVD (24, 25). Furthermore, obesity is also a risk factor for CVDs. Atherosclerosis, a complex process involving disruptions in lipid metabolism and chronic inflammation is considered central to the pathogenesis of CVD (26). Persistent elevation of total cholesterol, LDL cholesterol, and triglyceride levels in the blood contributes to the formation of atherosclerotic plaques, while higher levels of high-density lipoprotein (HDL) cholesterol appear to offer protection. Genetic diversity in genes responsible for apolipoproteins, enzymes, and hormones can influence an individual's susceptibility to CVD. Some of these genetic variants are responsive to dietary modifications. For instance, individuals carrying the E4 allele in the apolipoprotein E gene tend to have higher levels of LDL cholesterol compared to those with other alleles (E1, E2, and E3) (27). Apolipoprotein A1 (ApoA1), mainly present in HDL particles, is associated with HDL-cholesterol concentration (28).

Notably, research by Ordovas et al. (29) demonstrated that the A allele was correlated with reduced serum HDL levels. Interestingly, this genetic effect was reversed in women who consumed more polyunsaturated fatty acids (PUFA). In men, this relationship was more pronounced when accounting for alcohol consumption and smoking. For instance, a specific polymorphism in the hepatic lipase gene is linked to increased protective HDL levels when compared to the TT genotype (more common in certain ethnic groups, like African–Americans), particularly in response to a high-fat diet (30). A study by Estruch et al. reported that a PUFA-enriched diet (such as extra virgin olive oil) can decrease the low-density lipoprotein receptor-related protein (LRP1) gene expression (31). Similarly, another study by Llorente-Cortés et al. showed that dietary components can modulate the expression of pro-atherothrombotic genes in susceptible individuals (32). Apart from obesity and CVDs, the role of various nutritional factors is well-established in other conditions including polycystic ovary syndrome, retinal diseases, lymphedema and COVID-19 (33-39).

Single Nucleotide Polymorphisms (SNPs) and Metabolic Significance

At the heart of nutrigenomics lie single nucleotide polymorphisms (SNPs), the most common form of genetic variation among individuals (40). SNPs involve the substitution of a single nucleotide base at a specific position in the DNA sequence. While most SNPs have no discernible impact on health, some can significantly affect the function of proteins, enzymes, and other molecules involved in metabolism. These functional SNPs can contribute to variations in nutrient metabolism and response to dietary components. Previously, Ames highlighted the critical role of this genetic diversity in influencing the individualized requirements for nutrients and subsequent physiological responses (41). Within expressed genes, missense single nucleotide polymorphisms are encountered at an approximate frequency of 1 in every 1000 bases (42). Therefore, it is reasonable to anticipate an abundance of additional polymorphisms that will emerge from studies focused on micronutrients and dietary behaviors. Each gene can be likened to a recipe dictating the synthesis of particular proteins or protein groups. These proteins either orchestrate pivotal biological processes or serve as the foundational constituents of bodily structures like collagen. Some SNPs introduce modifications to the gene's recipe, leading to either the production of a distinct protein quantity or the transformation of the structural arrangement of protein molecules (43).

Aim of the review

Currently, there is a lack of literature that provides comprehensive evidence on SNPs related to lipid and carbohydrate metabolism. This review aims to provide the current body of evidence regarding the latest developments regarding SNPs in lipid and carbohydrate metabolism.

Methodology

For this review, a comprehensive search was carried out in PubMed and Google Scholar to find relevant studies on the topic. We used Boolean operators AND and OR to combine appropriate keywords. The search terms used included "Nutrigenomics" OR "Nutrient-gene interactions" OR "Nutrigenetic variations" AND "Lipid metabolism" OR "Fatty acid metabolism" OR "Cholesterol metabolism" AND "Carbohydrate metabolism" OR "Glucose metabolism" AND "Single Nucleotide Polymorphisms" OR "Genetic variations" OR "Polymorphic genes". The study selection was limited to meta-analyses, multicenter studies, reviews, systematic reviews, observational studies, case-control studies, longitudinal/prospective studies, retrospective studies, and randomized controlled trials. We further refined our search by limiting the publication to English studies only. Furthermore, texts available only in abstract form were excluded.

Results and Discussion

Polymorphisms and Their Possible Effects on Nutrition

Polymorphisms play a key role in establishing how our bodies absorb, metabolize, and respond to nutrients. These variations can ultimately lead to the development of health-related conditions. Genetic polymorphisms linked to diverse metabolic pathways have been investigated through genome-wide association studies (44). Furthermore, the connections between genetic variations and dietary consumption have been explored through epidemiological and interventional research (45). For example, some instances of such associations include 1) the relationship between the APOA2 (c.2265T>C) variant and saturated fatty acid intake, as well as body mass index; 2) the correlation between MTHFR variants and homocysteine levels; 3) the link between CYP1A2 variants and the hypertensive response triggered by caffeine (46, 47). The genomic revolution of the past few decades has propelled our understanding of genetics to unprecedented heights. The mapping of the human genome, launched in 1990 and finished in 2003 opened doors to a treasure trove of information about our genetic blueprint. Within this genetic diversity lies the key to comprehending why some people thrive on certain diets while others struggle, and why certain nutritional interventions yield remarkable results for some but not for others (48, 49).

The influence of polymorphisms on nutrition is multifaceted: for instance, the well-known MTHFR gene variant affects the enzyme responsible for converting folate into its active form, which is crucial for various biological processes, including DNA synthesis and repair (50). Individuals with this polymorphism may have an impaired ability to metabolize folate, which makes them more susceptible to certain health issues, like neural tube defects and cardiovascular diseases. MTHFR has been associated with increased breast cancer risk in individuals with reduced intake of vitamin B6, vitamin B12, and folate. However, a review by Chen et al. has reported that MTHFR C677T gene polymorphism is associated with breast cancer risk among Asians, but not Caucasians (51). Understanding these genetic nuances is crucial for tailoring personalized nutrition recommendations.

Carbohydrates, Their Metabolism, and Their Role

Among the essential macronutrients, carbohydrates emerge as a focal point of this intersection between nutrition and genetics. Their metabolism within the human body plays a pivotal role in energy production, cellular function, and disease susceptibility. Carbohydrates have long held the limelight in the realm of nutrition. From ancient civilizations subsisting on grains to modern diets shaped by cultural and industrial shifts, they have been a dietary staple. Comprising sugars, starches, and fibers, carbohydrates serve as a primary source of energy for the human body (52).

While the fundamental role of carbohydrates as an energy source is widely acknowledged, the interplay between carbohydrate consumption, genetic makeup, and health outcomes has only recently been illuminated through the lens of nutrigenomics. The journey begins with the digestion of complex carbohydrates into simpler sugars, such as glucose. The roles of carbohydrates extend beyond their energy-providing function: a prime example are dietary fibers, a subset of carbohydrates that is indigestible by human enzymes but act as substrates for gut microbiota. This symbiotic relationship between the gut microbiome and dietary fibers underscores the emerging concept of "second genome," where microbial genes actively interact with our human genes to influence metabolism, immunity, and disease susceptibility (53). Nutrigenomics research has demonstrated how certain genetic variants impact the body's response to dietary fibers, potentially affecting the microbial composition of the gut and subsequently influencing an individual's risk of obesity, inflammatory disorders, and even mental health conditions (54).

Lipids, Their Metabolism, and Their Role

One area of particular interest within nutrigenomics is the study of lipids—a diverse group of organic molecules that play fundamental roles in cellular structure, energy storage, and signaling. Understanding lipid metabolism and its role in human physiology is essential for uncovering the intricate connections between our dietary habits, genetic predispositions, and the development of various health conditions. Lipids, encompassing diverse molecules such as fatty acids, triglycerides, phospholipids, and cholesterol, serve as the structural foundation of cellular membranes, contributing to their integrity and fluidity. Central to the

Table 1. Carbohydrate-Related Genes, Their Polymorphisms, and Their Association with Metabolic Traits and Obesity-Related Risks

RsID	Gene	Polymorphism function	Alleles	wt/mt	References
rs266729	ADIPOQ	Diminished hormone levels	G/G	mt/mt	(55)
		Diminished hormone levels	C/G	wt/mt	
		Typical	C/C	wt/wt	
rs2167270	LEP	Risk of high BMI and insulin resistance	A/A	mt/mt	(55)
		Risk of high BMI and insulin resistance	G/A	wt/mt	
		Typical	G/G	wt/wt	
rs7799039	LEPR	Increased risk of high BMI	A/A	mt/mt	(56, 57)
		Increased risk of high BMI	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs5219	KCNJ11	Impaired glucose-induced insulin secretion with high BMI Greater impairment of insulin release	T/T	mt/mt	(58, 59)
		Impaired glucose-induced insulin secretion with high BMI	C/T	wt/mt	
		Typical	C/C	wt/wt	
rs11185098	AMY1	Lower amylase activity Bad at breaking down carbs	A/A	mt/mt	(60)
		Intermediate amylase activity Still good at breaking down carbs	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs659366	UCP2	Increased risk of higher BMI	T/T	mt/mt	(61-65)
		Increased risk of higher BMI	C/T	wt/mt	
		Typical	C/C	wt/wt	
rs1800849	UCP3	Lower glucose levels Better weight loss on high protein/low carb diet	A/A	mt/mt	(66)
		Less weight loss No decrease in glucose or insulin levels on high protein/low carb diet	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs1801282	PPARγ2	Increased risk of insulin resistance	G/G	mt/mt	(67, 68)
		Increased risk of insulin resistance	C/G	wt/mt	
		Typical	C/C	wt/wt	
rs116987552	PYGM	Absence of the enzyme	A/A	mt/mt	(69)
		Deficiency of the enzyme	G/A	wt/mt	
		Typical	G/G	wt/wt	

study of nutrigenomics is the realization that our genetic makeup influences how our bodies interact with and respond to dietary components, including lipids. Genetic variations can impact enzymatic activities involved in lipid metabolism, affecting the way we process and utilize dietary lipids. For example, certain individuals may possess genetic variants that lead to decreased activity of enzymes responsible for breaking down specific types of dietary fats (70). As a result, these individuals might have a higher risk of accumulating excess fat and facing associated health challenges.

The influence of dietary lipids on gene expression adds another layer of complexity to the nutrigenomic landscape. Emerging research suggests that dietary lipids can act as signaling molecules, modulating gene expression and influencing metabolic pathways. Omega-3 and omega-6 fatty acids, for instance, have been shown to regulate the expression of genes involved in inflammation, lipid oxidation, and insulin sensitivity (71, 72). Variations in lipid metabolism genes can impact the production of these lipid mediators, influencing an individual's susceptibility to chronic inflammatory conditions and cardiovascular diseases.

SNPs Correlated to Macronutrients

The food we consume not only provides our body with the energy to function, but also act as a foundation for our overall well-being. However, the impact of nutrition goes beyond the generic understanding of food as mere sustenance. Macronutrients, including lipids and carbohydrates, play a crucial role in energy production, growth, and overall health. The efficiency of our body's metabolism is influenced by genetic factors, which can vary significantly among individuals due to SNPs. Individuals with different genetic profiles respond differently to macronutrient intake. Studying SNPs allows us to understand how an individual's genetics might impact their ability to metabolize and utilize lipids and carbohydrates. This knowledge can lead to personalized dietary recommendations that optimize health outcomes. Certain genetic variations can increase the susceptibility to metabolic disorders, such as obesity, type 2 diabetes, and cardiovascular diseases. Identifying and understanding SNPs associated with these conditions can aid in early disease detection and prevention. Knowledge of genetic variations

Table 2. Lipid-Related Genes and Their Polymorphisms

RsID	Gene	Function	Alleles	wt/mt	References
rs174547	FADS1	Decreased fatty acid desaturase enzyme activity	C/C	mt/mt	(55)
		Decreased fatty acid desaturase enzyme activity	T/C	wt/mt	
		Typical	T/T	wt/wt	
rs1558902	FTO	Risk for high BMI, but not associated with problems related to obesity Better response to high-protein diets	A/A	mt/mt	(73-76)
		Somewhat increased risk for high BMI	A/T	wt/mt	
		Typical	T/T	wt/wt	
rs5082	APOA2	Increased risk of high BMI, particularly with diets rich in saturated fats	G/G	mt/mt	(40, 77-79)
		Typical	A/G	wt/mt	
		Typical	A/A	wt/wt	
rs662799	APOA5	32% increase in triglyceride levels	G/G	mt/mt	(40, 77, 80-82)
		16% increase in triglyceride levels	A/G	wt/mt	
		Typical	A/A	wt/wt	
rs5128	APOC3	Higher fasting plasma levels of APOC3, TG, TC and LDL-C	G/G	mt/mt	(40, 77, 83)
		Higher fasting plasma levels of APOC3, TG, TC and LDL-C	C/G	wt/mt	
		Typical	C/C	wt/wt	
rs2070895	LIPC	Significantly higher HDL-C level	G/G	mt/mt	(77, 84, 85)
		Significantly higher levels of FPG, TC, TG	G/A	wt/mt	
		Significantly higher levels of FPG, TC, TG	A/A	wt/wt	
rs987237	TFAP2B	Better response to high-protein diets for weight management	A/A	mt/mt	(84, 86)
		Typical	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs11591147	PCSK9	Decreased LDL-cholesterol	T/T	mt/mt	(87-90)
		Decreased LDL-cholesterol	G/T	wt/mt	
		Typical	G/G	wt/wt	
rs72646508	PCSK9	Decreased LDL	T/T	mt/mt	(87, 88, 91)
		Decreased LDL	C/T	wt/mt	
		Typical	C/C	wt/wt	
rs505151 E670G	PCSK9	Increased LDL	G/G	mt/mt	(87, 88, 92-94)
		Increased LDL	A/G	wt/mt	
		Typical	A/A	wt/wt	
rs328	LPL	Lower triglycerides	G/G	mt/mt	(95-97)
		Lower triglycerides	C/G	wt/mt	
		Typical	C/C	wt/wt	
rs268	LPL	Higher triglyceride	G/G	mt/mt	(96, 98)
		Higher triglycerides	A/G	wt/mt	
		Typical	A/A	wt/wt	
rs1800592	UCP1	Weak protein activity Probable increase of abdominal fat and high BMI	C/C	mt/mt	(61, 99)
		Probably typical risk for high BMI	C/T	wt/mt	
		Typical	T/T	wt/wt	
rs3734398	ELOVL2	Decreased conversion of EPA to DHA	C/C	mt/mt	(100, 101)
		Decreased conversion of EPA to DHA	C/T	wt/mt	
		Typical	T/T	wt/wt	

can guide the development of targeted interventions and therapies for individuals with specific genetic predispositions, which could involve customized dietary plans or the use of specific medications to counteract the effects of certain SNPs.

Key Genes and SNPs in Lipid and Carbohydrate Metabolism

Several genes are known to harbor SNPs that influence lipid and carbohydrate metabolism. Some of the most important genes and SNPs in this context include:

ApoE Gene: The ApoE gene is associated with cholesterol metabolism and plays a role in lipid transport. Specific SNPs in this gene have been linked to variations in cholesterol levels and the risk of cardiovascular diseases. Furthermore, it has been shown that ApoE is linked with age-related risk for Alzheimer's disease and plays critical roles in Aβ homeostasis (102).

Pparg Gene: The Pparg gene is involved in regulating lipid and glucose metabolism. Certain SNPs in this gene can impact insulin sensitivity, lipid storage, and the risk of type 2 diabetes (103). A study by Hevener et al. reported that muscle-specific Pparg deletion resulted in insulin resistance in mice (104).

FTO Gene: The FTO gene is associated with obesity and appetite regulation. Variants of this gene have been shown to affect energy expenditure and the preference for high-calorie foods. A study by Hunt et al. reported that BMI increases associated with FTO genotypes begin in youth and are maintained throughout adulthood (105).

SLC2A2 Gene: This gene encodes a glucose transporter and is vital for glucose uptake into cells. SNPs in SLC2A2 can impact glucose homeostasis and the risk of diabetes.

LIPC Gene: The LIPC gene encodes an enzyme that plays a role in lipid metabolism. Certain SNPs in this gene are associated with variations in HDL cholesterol levels.

The field of nutrigenomics is still evolving, and there are several promising directions for future research. As we gather more data on the interaction between SNPs and macronutrient metabolism, we can develop more precise nutrition guidelines, tailored to an individual's genetic makeup. This could revolutionize dietary recommendations and improve health outcomes. While SNPs are important, epigenetic modifications – changes in gene expression without altering the DNA sequence – also play a role in macronutrient metabolism. Future research could focus on understanding how diet and lifestyle choices interact with genetic and epigenetic factors. Exploring how genetic variations and their effects on macronutrient metabolism differ among different ethnicities and geographical populations could provide a more comprehensive understanding of the complex interactions.

Conclusions

Each individual harbors a unique nutritional pattern, encoded within their genes. Bioactive compounds in food and essential nutrients wield an influence over how these genes are manifested. Significant evidence has demonstrated that food like the Mediterranean diet can influence the functioning of various genes. These diets can reduce inflammation and thus the progression of various chronic diseases. Studying the connection between SNPs and how our bodies process fats and carbohydrates has a lot of potential in the field of nutrigenomics. This could greatly improve how we understand individualized nutrition and taking care of our health. Figuring out how genes and our diet interact is important because it could lead to personalized advice on what to eat, spotting health issues early, and creating treatments that are tailored to each person. As scientists keep learning more, nutrigenomics could really change how we think about staying healthy in the future.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review

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Nutrigenomics: SNPs correlated to detoxification, antioxidant capacity and longevity

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Abstract

Nutritional genomics, also known as nutrigenomics, is the study of how a person's diet and genes interact with each other. The field of nutrigenomics aims to explain how common nutrients, food additives and preservatives can change the body's genetic balance towards either health or sickness. This study reviews the effects of SNPs on detoxification, antioxidant capacity, and longevity. SNPs are mutations that only change one nucleotide at a specific site in the DNA. Specific SNPs have been associated to a variety of biological processes, including detoxification, antioxidant capacity, and longevity. This article mainly focuses on the following genes: SOD2, AS3MT, CYP1A2, and ADORA2A (detoxification); LEPR, TCF7L2, KCNJ11, AMY1, and UCP3 (antioxidant capacity); FOXO3 and BPIFB4 (longevity). This review underlines that many genes—among which FOXO3, TCF7L2, LEPR, CYP1A2, ADORA2A, and SOD2—have a unique effect on a person's health, susceptibility to disease, and general well-being. Due to their important roles in numerous biological processes and their implications for health, these genes have undergone intensive research. Examining the SNPs in these genes can provide insight into how genetic variants affect individuals' responses to their environment, their likelihood of developing certain diseases, and their general state of health. ***Clin Ter 2023; 174 Suppl. 2 (6):209-213 doi: 10.7417/CT.2023.2489***

Key word: *SNPs, Nutrigenomics, longevity, detoxification, antioxidant*

Introduction

Nutrigenomics

Nutritional genomics is an emerging area of study that integrates the domains of nutrigenetics and nutrigenomics (1,2). Nutrigenomics holds the promise of yielding notable health advantages. However, despite the demand for medical geneticists being substantial, their availability is still limited. Furthermore, primary care physicians possess only basic training in nutrition and genetics (3). It is predicted that the human genome polymorphisms map will contribute to establishing optimal diets and understanding the role that nutrition plays in human health and disease. Together, advanced genetic research and nutrigenomics studies may help us in learning how our unique genetic makeup contributes to the emergence of polygenic diet-related disorders, including cancer and cardiovascular diseases. With the ultimate goal of customizing food and nutrition based on an individual's genotype, nutrigenomics perceives food as a major environmental element in the gene-environment interaction (4).

In this study, detoxification, antioxidant capability, and longevity are discussed in relation to polymorphisms. Protecting against potentially hazardous chemicals and oxidative stress, detoxification plays a crucial role in maintaining overall health. The term "detoxification" refers to the physiological process, through which the body neutralizes potentially hazardous chemicals or toxins, or prepares them for elimination. The liver, kidneys, lungs, and skin are the key organs involved in detoxification (5). Cell damage, inflammation, and diseases are all possible outcomes of oxidative stress (6). Longevity in humans results from a

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dynamic interaction between genetic make-up and external stimuli. The heritability of longevity has been shown to be consistently low among many populations: both the aging and overall life span are heavily affected by our surroundings. To better inform public health efforts and promote population health, it is important to identify potential environmental determinants of longevity (7).

Polymorphisms and their possible effects on an individual's physiological status

Polymorphisms refer to variations in DNA sequence, the most prevalent kind of which is known as Single Nucleotide Polymorphism (SNP). Genetic variances can impact a person's disease risk, response to therapy, and overall health. There are several potential ways in which polymorphisms can affect physiological status; moreover, some polymorphisms have been related to an increased risk of contracting specific diseases (8). For example, the risk of getting breast and ovarian cancer is higher in people with certain SNPs in the BRCA1 and BRCA2 genes, immune system gene polymorphisms may contribute to the development of autoimmune disorders, and the way an individual reacts to a certain drug could be affected by a polymorphism. Polymorphisms have also been shown to alter metabolic pathways and the way nutrients are used by the body. Enzyme polymorphisms can result in a loss or gain of enzyme activity. This has the potential to influence numerous metabolic pathways and activities (9).

Health outcomes may be affected by the interaction between genetic variants and environmental variables. For example, changes in genes involved in cellular repair, stress response, and metabolism can have an effect on how quickly or slowly aging occur. Moreover, the genetic make-up of some people may make them more resistant to the effects of particular environmental stresses or, on the other hand, particular SNPs are linked to elevated disease risks. The body's inflammatory and immunological responses can be affected by genetic differences, which in turn affects susceptibility to infections and autoimmune diseases (10).

In short, health outcomes are determined by a complex relationship of genetic predisposition, environmental variables, and behavioral choices. Not all polymorphisms have clearly defined impacts, either, and genetics as a whole is always developing, as new studies reveal previously unknown factors that affect human health (11,12).

SNPs influence on detoxification and antioxidant capacity

Different biological functions, such as detoxification, antioxidant capability, and longevity, have been linked to particular SNPs. Genetics is simply one of the many factors that determine these characteristics, and our understanding of genetics is expanding all the time. Toxic chemicals are eliminated from the body by a physiological process, known as metabolic detoxification (13,14). The sensitivity of the body to both endogenous and exogenous toxins may be influenced by genetic variability and dietary variables, both of which can affect the action of detoxification enzymes (13,14). The influence of particular SNPs on detoxification and antioxidant capacity can differ significantly, depending on the gene expression and some environmental context. (13,15).

The process of detoxification is how the body breaks down and gets rid of toxic components, including poisons, medications, and pollution. By changing the activity of the enzymes responsible metabolizing and removing these chemicals, SNPs can impact the detoxification pathways. The cytochrome P450 (CYP) enzyme family, which helps

Table 1. SNPs, their gene and function correlated to detoxification and antioxidants.

RsID	Gene	Function	Alleles	wt/mt	References
rs4880	SOD2	Enzyme activity enhanced (about 33% higher)	T/T	wt/wt	(18-21)
		Enzyme activity enhanced (about 33% higher)	C/T	wt/mt	
		Typical	C/C	mt/mt	
rs3740393	AS3MT	Faster and more effective arsenic detoxification	C/C	mt/mt	(22,23)
		Typical	C/G	mt/wt	
		Typical	G/G	wt/wt	
rs72547515	CYP1A2	Decreased activity or inactive enzyme	A/A	mt/mt	(24-27)
		Decreased enzyme activity	A/G	mt/wt	
		Typical	G/G	wt/wt	
rs762551	CYP1A2	Faster metabolism of caffeine	A/A	wt/wt	(24,28,29)
		Typical	A/C	mt/wt	
		Slower metabolism of caffeine	C/C	mt/mt	
rs2298383	ADORA2A	No increase in anxiety from caffeine (in average amount)	T/T	wt/wt	(24,30-32)
		No increase in anxiety from caffeine (in average amount)	C/T	mt/wt	
		Probable increase in anxiety from caffeine	C/C	mt/mt	

in the metabolization of several substances, contains some crucial genes involved in detoxification (16).For instance, an SNP might cause an enzyme to have lesser activity, which would result in delayed chemical detoxification. On the other hand, some SNPs can cause hyperactive enzymes, which may increase the likelihood of negative reactions to specific medications or chemicals (16).

Catalase and antioxidant enzymes' activities can be changed by SNPs in their respective genes. This may raise the risk of a number of conditions linked to oxidative damage, including cancer, neurological disorders, and cardiovascular diseases. Genetic investigations, such as Genome-Wide Association investigations (GWAS), are frequently used to identify specific SNPs that are connected to detoxification and antioxidant-related processes (17). Table 1 reports the SNPs that are correlated to detoxification and antioxidants.

Table 1 summarizes the impact of different genetic variations (SNPs) on particular genes and their functions. Changes in enzyme activity, metabolite levels, and reactions to caffeine or anxiety brought on by specific metabolites are among the main function of these SNPs. For instance, the presence of specific alleles is linked to either higher or lower SOD2 enzyme activity in the case of rs4880 in the SOD2 gene. Similar effects of the allele appear to affect urine excretion of arsenic metabolites for rs3740393 in the AS3MT gene.

The CYP1A2 gene has variants (rs72547515 and rs762551) that affect how caffeine is metabolized. The pace at which caffeine is metabolized by different alleles influences how quickly or slowly it is metabolized in the whole body. Anxiety responses to coffee are linked to the rs2298383 SNP in the ADORA2A gene. Despite consuming large amounts of caffeine, some genotypes manifest no increase in anxiety, while others imply that large amounts of caffeine may increase anxiety. The explanations given here are based on the data in Table 1; nevertheless, for a more thorough understanding, it is advised to consult relevant scientific literature and molecular biology and genetics specialists (32).

SNPs influence on longevity

Research on increasing life expectancy is more commonly associated with the term "longevity," which can be alternatively thought of as "maximal lifespan". Life expectancy is affected by both genetics and environmental variables. As far as genetics are involved, longevity is likely affected by at least three distinct groups of SNPs. First of all, numerous SNPs are thought to affect susceptibility to diseases that shorten life expectancy. The second group is comped by SNPs that are common among elder people, thus appearing to increase longevity. Third, some SNPs may have an effect on one's lifespan only in specific environmental settings, such as those that shorten or increase lifespan only in people with a specific genotype who are also exposed to specific foods or pollutants (32). Table 2 reports the SNPs correlated to longevity.

Table 2 provides a list of genetic variations (RsIDs), their associated genes, and their effects. It is concluded that the genes FOXO3 and BPIFB4 play roles in longevity or long-lived qualities. The characteristics include those that increase the likelihood of living a long life and extending average or normal longevity. FOXO3 is a transcription factor and a member of the FOXO family, sharing the FHRE DNA consensus sequence. The FOXO3 SNP rs2802292 minor G-allele is closely linked to human longevity, and its copy number demonstrated a favorable correlation with decreased susceptibility to age-related diseases. If the BPIFB4 gene's rs2070325 is connected to "long-lived variant" and "typical longevity," this could imply that this genetic variation is linked to longer lifespans. It's crucial to remember that environmental conditions, interactions with other genes, and lifestyle choices can all have an impact on how genes affect longevity (33).

Discussion

Why study SNPs correlated to detoxification, antioxidant capacity and longevity

SNPs that are associated with lifespan, antioxidant capacity, and detoxification are an interesting subject of study, to learn more about numerous aspects of human health and ageing (33). For example, SNPs can affect how a person metabolizes medications and pollutants: by understanding the SNPs associated with detoxification pathways, a person's genetic composition can be taken into account when designing medical treatments and drug dosages (39). Detoxification pathways are essential for the body to digest and get rid of toxic chemicals, such as pollution, heavy metals, and carcinogens. Antioxidants are essential in the fight against oxidative stress, which has been related to ageing and a number of chronic diseases. Researchers can learn more about why certain people are more prone to oxidative damage and age-related disorders than others by studying SNPs that are associated with antioxidant enzymes and pathways. This information can direct interventions and way of life modifications to support better health and lifespan. Finding lifespan-linked SNPs can reveal information about the genetic causes of a longer, healthier life. By examining

Table 2. SNPs, their gene and function correlated to longevity.

RsID	Gene	Function	Alleles	wt/mt	References
		Increased odds of living longer	G/G	mt/mt	
		Increased odds of living longer	G/T	wt/mt	
rs2802292	FOXO3	Typical	T/T	wt/wt	(2,33-35)
		Long-lived variant	G/G	mt/mt	
		Typical longevity	A/G	wt/mt	
rs2070325	BPIFB4	Typical	A/A	wt/wt	(36-38)

these genetic markers, scientists can identify the processes and mechanisms that support healthy ageing and create interventions to support it (40).

Risks of low detoxification and antioxidant capacity

Having insufficient antioxidant and detoxifying ability might be harmful to one's health, since detoxification pathways and antioxidant systems are crucial in preventing oxidative stress, environmental contaminants, and the accumulation of dangerous chemicals in cells and tissues. Cellular damage, inflammation, and the emergence of several chronic diseases and neurological problems have all been linked to oxidative stress. Oxidative stress is also a significant contributor to ageing. A compromised detoxification system could result in the body storing harmful substances, which, together with oxidative stress, can harm the immune system's response. Moreover, cardiovascular diseases are often accompanied with inflammation and oxidative stress, because a decreased antioxidant capacity can raise the risk of cardiovascular issues. A low capacity for detoxification can make the breakdown and removal of toxins ineffective. Genetics, lifestyle choices, and environmental exposure all have a role in a person's capacity for detoxification and antioxidant defense (41).

Most important genes having SNPs involved in these mechanisms

This review would like to underline the importance of the genes SOD2, AS3MT, CYP1A2, and ADORA2A, which are involved in detoxification; genes LEPR, TCF7L2, KCNJ11, AMY1, and UCP3, concerning antioxidant capacity; and finally, the aforementioned FOXO3 and BPIFB4, which can affect longevity. This study came to the conclusion that all of these genes are significant, because they each have a unique effect on a person's health, susceptibility to disease, and general well-being. For example, longer lifespans and a lower chance of developing age-related disorders have been associated with variations in gene FOXO3. One of the most significant genes associated with the risk of type 2 diabetes is TCF7L2, whose variations significantly impact the control of insulin and glucose metabolism. Gene LEPR plays a role in metabolism and appetite control (41), so its SNPs might impact an individual's antioxidant capability and susceptibility to illnesses caused by oxidative damage. Due to their important roles in many biological processes and their implications for health, these genes have undergone intensive research (42). Examining these genes' SNPs can provide insight into how genetic variants affect how individuals respond to their environment, their likelihood of developing certain diseases, and their general state of health (43).

Outlook for Future Research

This review suggests that many genes and SNPs have a positive or negative effect on longevity and detoxification or antioxidant capacity, resulting in multiple effects on our organism. Future studies in nutrigenomics with a focus on SNPs linked to antioxidant capacity, detoxification, and lifespan have a great deal of potential to further extend our knowledge of individualized medicine, disease prevention, and ageing control. The study of SNPs in different cohorts could also be important to identify possible differences in detoxification, antioxidant capacity, and longevity in different populations.

Conclusions

Nutritional genomics is a new field of study, combining the former distinct fields of nutrigenetics and nutrigenomics. The key research areas include gene expression, protein and metabolite concentration, and consequently metabolism, health state, and disease risk. This covers the effects of dietary non-nutritive bioactive substances like enzyme inhibitors. Nutrigenomics may result in major potential health advantages. Currently, medical geneticists are in high demand, and yet are hard to come by because primary care doctors only have a basic understanding of nutrition and genetics. This study examines the effects of SNPS on detoxification, antioxidant capacity, and longevity, coming to the conclusion that SNPs are mutations that only affect one nucleotide at a specific site in the DNA. Specific SNPs have been associated to a variety of biological processes, including detoxification, antioxidant capacity, and longevity. This study concluded that all of these genes—FOXO3, TCF7L2, LEPR, CYP1A2, ADORA2A, and SOD2—are significant, because they each have a unique effect on a person's health, susceptibility to disease, and overall well-being.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review

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Nutrigenomics: SNPs correlated to Food Preferences and Susceptibilities

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Abstract

Background.Nutrigenomics explores the intricate interplay between single nucleotide polymorphisms (SNPs), food preferences, and susceptibilities.

Methods. This study delves into the influence of SNPs on food sensitivities, allergies, tyramine intolerance, and taste preferences. Genetic factors intricately shape physiological reactions to dietary elements, with polymorphisms contributing to diverse sensitivities and immune responses.

Results. Tyramine intolerance, arising from metabolic inefficiencies, unveils genetic markers exerting influence on enzyme function. SNPs transcend genetic diversity by exerting substantial impact on food sensitivities/allergies, with specific variants correlating to heightened susceptibilities. Genes accountable for digesting food components play pivotal roles. Given the rising prevalence of food sensitivities/allergies, understanding genetic foundations becomes paramount. In the realm of taste and food preferences, SNPs sculpt perception and choice, yielding variances in taste perception and preferences for sweetness, bitterness, and umami. This genetic medley extends its reach to encompass wider health implications.

Conclusions. In this review article, we have focused on how polymorphisms wield significant sway over physiological responses, sensitivities, and dietary inclinations. Unraveling these intricate relationships illuminates the path to personalized nutrition, potentially revolutionizing tailored recommendations and interventions. ***Clin Ter 2023; 174 Suppl. 2 (6):214-226 doi: 10.7417/CT.2023.2490***

Key word: *Nutrigenomics, SNPs, food preferences, food sensitivities, taste, genetics*

Introduction

The fascinating landscape of nutrigenomics, a dynamic field at the intersection of genetics and nutrition, unravels the intricate correlations between genetic variations, physiological responses, and nutritional status (1). Subtle genetic changes known as single nucleotide polymorphisms (SNPs), hold the potential to exert profound effects on our physiological and nutritional well-being, shaping our susceptibility to various health conditions and influencing our dietary choices (2-4). Among the multifaceted phenomena that nutrigenomics seeks to illuminate are food sensitivities, food allergies, and tyramine intolerance (5). Food sensitivities and allergies represent intricate reactions to specific dietary components, which, in contrast to tyramine intolerance, involve immune system responses (6). Tyramine intolerance, on the other hand, is characterized by the body's inability to efficiently metabolize tyramine, a compound naturally occurring in certain foods (7).

SNPs, the genetic orchestrators, weave a complex tapestry that can influence the development of food sensitivities, allergies, and tyramine intolerance (8). These genetic variations can contribute to altered physiological responses to specific dietary constituents, resulting in adverse reactions like gastrointestinal discomfort, bloating, or more severe allergic responses (9). The connection between genetic variations and tyramine intolerance is equally captivating (10): as tyramine intolerance arises from inefficient tyramine metabolism, SNPs can play a pivotal role in shaping an individual's capacity to metabolize this compound (7). The resulting accumulation of tyramine can trigger adverse symptoms, ranging from headaches to migraines (11).

Beyond these physiological intricacies, SNPs also extend their influence to the captivating realms of taste and food

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preferences (12). Genetic variations impact our ability to perceive taste qualities such as sweetness, bitterness, and umami, thereby influencing our inclinations towards certain foods (13). This genetic symphony guides our dietary choices, as individuals with specific SNPs may exhibit heightened sensitivity to particular taste profiles, influencing their nutritional behaviors (14). In this exploration of nutrigenomics, we embark on a journey that delves into the depths of genetic polymorphisms and their potential ramifications on physiological responses and nutritional well-being (15). We peel back the layers to uncover the mechanisms through which SNPs can influence the development of food sensitivities, allergies, and tyramine intolerance, shedding light on the intricate interplay between genetics and dietary reactions (8).

Nutrigenomics can help us understanding how SNPs shape an individual's propensity to encounter adverse reactions to specific foods, unraveling the delicate balance between genetics and dietary choices (16-18). This multidimensional discipline promises not only a deeper understanding of the intricate connections between genetics and nutrition, but also holds the potential for the development of personalized dietary recommendations resonating with our unique genetic makeup (19). The captivating landscape of nutrigenomics has the potential to enable us to decode the genetic blueprints governing our dietary experiences, ultimately empowering us to make informed choices for our nutritional well-being.

Polymorphisms: Unraveling their Influence on Physiological and Nutritional Status

Genetic polymorphisms, variations in the DNA sequence that are present in a population, form the bedrock of human genetic diversity (20). These tiny deviations can give rise to diverse phenotypic outcomes, dictating responses to external stimuli, including nutrients (21): polymorphisms can modulate enzymatic activities, receptor sensitivities, and signaling pathways, which in turn affect how the body interacts with nutrients (22). Such genetic variability is key in shaping the wide spectrum of individual responses to diet, from nutrient metabolism to susceptibility to various health conditions (23).

The influence of polymorphisms on one's physiological status is exemplified in their effects on nutrient metabolism (16): for instance, genetic variations within genes responsible for lipid metabolism can influence an individual's predisposition to obesity, dyslipidemia, and related cardiovascular diseases (24). Research has identified polymorphisms in genes, like FTO (fat mass and obesity-associated), that are associated with differences in energy expenditure and fat storage (25). Similarly, polymorphisms in the FADS1/FADS2 gene cluster impact the conversion of essential fatty acids, showcasing how genetic variability can sway the body's handling of nutrients and impact overall physiological status (26).

Micronutrients, vital molecules for various physiological processes, are not exempt from the influence of polymorphisms (27). Genetic variations can modulate the absorption, transport, and utilization of micronutrients, leading to varying nutritional statuses (28). A case in point is the MTHFR gene, which encodes an enzyme involved in folate metabolism (29). Polymorphisms in MTHFR have been linked to altered folate metabolism and increased susceptibility to neural tube defects (30). Such insights into the intricate interplay of genetics and micronutrient metabolism emphasize the importance of considering genetic factors in tailoring dietary recommendations (31).

As genetic research advances, the horizon of polymorphism research expands (32). The advent of genome-wide association studies (GWAS) has enabled the identification of numerous genetic variants associated with various physiological and nutritional outcomes (33). However, challenges remain in deciphering the functional implications of these polymorphisms and translating them into actionable insights for personalized health management (34).

Food Sensitivities and Allergies: Navigating the Terrain of Dietary Reactions

The journey of dietary intake can complex, marked by a variety of responses that range from enjoyment to potential adverse reactions (5). Among these, food sensitivities and allergies stand out as intricate phenomena that can significantly impact an individual's health and well-being (35).

Food sensitivities encompass a spectrum of adverse reactions to certain foods that do not involve the immune system's immediate response, as seen in allergies (36). These reactions often manifest as gastrointestinal discomfort, bloating, indigestion, or vague symptoms like fatigue or headaches (37). Unlike allergies, which are characterized by immune-mediated responses, sensitivities can stem from various factors, such as enzyme deficiencies or non-immune mechanisms (38). Lactose intolerance, for example, arises due to insufficient lactase enzyme activity required to digest lactose in dairy products (39). Similarly, non-celiac gluten sensitivity presents with symptoms similar to celiac disease, but lacks the autoimmune and intestinal damage components (40). In contrast to sensitivities, food allergies evoke an immune-mediated response that can range from mild discomfort to severe, life-threatening reactions (41). When the immune system perceives certain food proteins as threats, it mounts an attack, releasing histamines and other chemicals that trigger symptoms (42): a prime example is peanut allergies, which can lead to anaphylaxis, a severe reaction that compromises breathing and requires immediate medical attention (43). The complexity of allergies lies in their potential to emerge suddenly, even after years of uneventful consumption of the allergenic food.

Both food sensitivities and allergies are influenced by genetic and environmental factors (44). Genetic predisposition can determine an individual's susceptibility to certain reactions (45). Polymorphisms in genes responsible for digesting specific food components, such as lactase for lactose or HLA-DQ2/DQ8 for celiac disease, can contribute to an increased likelihood of developing sensitivities or allergies (46). The prevalence of food sensitivities and allergies is on the rise, a trend that has perplexed researchers and healthcare professionals alike (5). Factors such as changes in dietary habits, environmental exposures, and gut microbiome alte-

rations have been proposed as potential contributors (47). Moreover, the so-called "hygiene hypothesis" suggests that reduced exposure to microbes in early childhood could lead to an exaggerated immune response to harmless substances, potentially increasing the risk of allergies (48).

Understanding the nuances of food sensitivities and allergies has significant implications for personalized nutrition and healthcare (49). Accurate diagnosis is paramount to guiding dietary choices and minimizing adverse reactions (50). Genetic testing can offer insights into an individual's predisposition to certain sensitivities or allergies, allowing for tailored dietary recommendations (51). Moreover, integrating genetic information with an individual's medical history and symptoms can refine the diagnostic process and guide appropriate interventions. Table 1 shows studies used to establish genetic polymorphism involved in food sensitivities and allergies.

Various investigations on genetic influences on autoimmune disorders and allergies have uncovered an intricate landscape. Lessard et al. delved into Sjögren's syndrome, uncovering significant associations with genes involved in innate and adaptive immunity, exemplified by the HLA region at 6p21 and other key loci (57). Shifting focus to food allergies, a pioneering study explored the genetic underpinnings of hydrolyzed wheat protein (HWP) allergy, shedding light on genetic associations within the HLA region and RBFOX1 locus, highlighting the complexity of susceptibility (55). With food allergies affecting a substantial proportion of children, a comprehensive review sought to elucidate the intricate interplay between human leucocyte

Table 1. SNPs for Food Sensitivities and Allergies

RsID	Gene	Function	Alleles	wt/mt	References
rs2187668	HLA-DQA1	Typical	C/C	wt/wt	(52-54)
		Gluten intolerance is possible	C/T	wt/mt	
		Gluten intolerance is possible	T/T	mt/mt	
rs9271588	HLA-DQA1	Higher risk of wheat allergy	C/C	mt/mt	(52,55-57)
		Typical	C/T	wt/mt	
		Typical	T/T	wt/wt	
rs9275596	HLA-DQB1/DQA2	3-fold increase of the relative risk of peanut allergy in Caucasians	C/C	mt/mt	(58-60)
		1.7-fold increase of the relative risk of peanut allergy in Caucasians	C/T	wt/mt	
		Typical	T/T	wt/wt	
rs20541	IL13	Typical	G/G	wt/wt	(61-67)
		Higher IgE levels; higher risk of allergies, allergic rhinitis; increased risk of dust mite and shrimp allergies	A/G	wt/mt	
		Higher IgE levels; higher risk of allergies, allergic rhinitis; increased risk of dust mite and shrimp allergies	A/A	mt/mt	
rs1800925	IL13	Typical	C/C	wt/wt	(62, 64, 68-71)
		Increase IgE levels; typical risk of shrimp allergy	C/T	wt/mt	
		Increase IgE levels; increased risk of shrimp allergy	T/T	mt/mt	
rs1295686	IL13	Typical	C/C	wt/wt	(64, 72-74)
		Increased risk of food allergies; elevated plasma IgE	C/T	wt/mt	
		Increased risk of food allergies; elevated plasma IgE	T/T	mt/mt	
rs917997	IL-18	Slightly increased relative risk of gluten intolerance	T/T	mt/mt	(75,76)
		Slightly increased relative risk of gluten intolerance	C/T	wt/mt	
		Typical	C/C	wt/wt	
rs2243250	IL-4	Increased risk of food allergies in conjunction with vitamin D deficiency (most common genotype in Caucasians)	C/C	mt/mt	(77-79)
		Increased risk of food allergies in conjunction with vitamin D deficiency	C/T	wt/mt	
		Food allergy risk not dependent on vitamin D levels (most common genotype in Asian and African populations)	T/T	wt/wt	

antigen (HLA) genes and food allergy development, providing valuable insights into the origins and outcomes of this complex disorder (58).

In-depth genome-wide association studies honed in on specific allergies (such as peanut allergy), revealing significant loci within the HLA-DR and -DQ gene region and emphasizing the pronounced genetic risk posed by these genes (59). However, the genetic basis of peanut allergy is multifaceted, as a study of sibling pairs with discordant peanut allergies revealed associations of HLA class II alleles with the condition, albeit with complex statistical implications (60). Exploring broader respiratory and allergic conditions, longitudinal studies delved into the impact of genetic variants in IL13 and IL4 on asthma and allergy susceptibility, revealing associations and interactions that provide nuanced insights into disease development (61). Meta-analyses further highlighted significant associations between IL-13 polymorphisms and increased asthma risk, emphasizing the role of genetic variations across diverse populations (62). Antibiotic exposure's complex interaction with genetic risk was investigated in the context of atopic dermatitis (AD), showcasing the interplay between environment and genetics in disease development (63). Moving into chronic obstructive pulmonary disease (COPD) and bullous pemphigoid (BP), studies delved into the role of IL-13 gene polymorphisms, revealing associations with disease risk and potential therapeutic implications (64, 65). Expanding the scope to encompass autoimmune conditions like alopecia areata (AA), researchers confirmed and identified susceptibility loci, linking key genes such as IL-13 and KIAA0350/CLEC16A to AA susceptibility and broader autoimmune trends (66). Further unraveling the genetic underpinnings of allergic rhinitis (AR), investigations into IL-13 and IL-4 polymorphisms provided insights into their co-association with AR and sensitivity to aeroallergens, sparking the need for deeper cross-population investigations (67).

The genetic underpinnings of asthma, a complex hereditary respiratory disorder, have been a subject of intensive investigation. Research by Wang et al. unveiled a significant association between the IL-13 +1923C/T polymorphism and asthma risk, particularly in Asians and Caucasians. This genetic variant not only correlated with asthma susceptibility, but also exhibited links to elevated IgE levels, implicating its role in asthma development (68). Extending the exploration of genetic factors related to allergic responses, Granada et al. conducted a comprehensive genome-wide association study (GWAS) that illuminated thirteen significant SNPs within FCER1A, STAT6, and IL-13 genes, shedding light on genetic factors contributing to IgE dysregulation and clinical atopy (69). Zhang et al. delved into the realm of periodontitis susceptibility, revealing associations between the IL-13 -1112C/T polymorphism and this condition, particularly in chronic periodontitis (CP). However, further studies are needed to corroborate these findings across larger and more diverse cohorts (70).

In the context of shrimp allergy, Laha et al. discovered that specific genetic risk genotypes of HLA-DQ and IL13 polymorphisms were significantly linked to challenge-positive shrimp allergy, underlining the role of genetics in allergic reactions (71). Sadeghnejad et al. illuminated the dynamic interplay between genetics and environment in childhood wheezing and asthma, emphasizing the impact of maternal smoking and gene interactions in influencing disease outcomes (72). Al-Sayed et al. focused on bronchial asthma and the IL-13 gene polymorphism R130Q in Egyptian children, uncovering associations between this variant, serum IgE levels, and allergic inflammation, potentially pointing toward gene therapy avenues (73). Matysura et al. further explored genetic links to allergies, identifying significant associations between IL-4 and IL-13 genotypes and cow's milk allergy (CMA) risk in Ukrainian children (74). Ashley et al. delved into the realm of food allergies, uncovering consistent associations between IL13 genetic polymorphisms and challenge-proven food allergy, suggesting a potential role for this gene variant in IgE-mediated food allergy development (75). Expanding the genetic landscape, Hunt et al. conducted a genome-wide association study for celiac disease, identifying risk variants in immune response genes like IL12A and further highlighting the interconnectedness of immune-related genes in different autoimmune conditions (76).

The implications of vitamin D deficiency (VDD) in allergy risk were also explored, revealing the influence on IgE levels of specific genetic variations in genes like FCER1A, IL13, and IL4. The diverse ethnic prevalence of risk alleles underscored the intricate role of genetics in allergy outcomes (78). As allergic diseases continue to pose a growing health concern, IL-4's pivotal role in immune response and IgE production was highlighted as a central player in allergic disease development (79).

Food Intolerance

Food intolerance, distinct from allergies, encompasses adverse reactions to certain foods due to genetic markers, rooted in impaired digestion or metabolism. This complex interplay between genetics and dietary responses has been explored in various contexts. Dzialanski et al. investigated the traditional view of lactose intolerance, revealing an intermediate phenotype. Their oral lactose load test study found higher hydrogen levels in exhaled air for the CT genotype, suggesting a genetic influence on lactose metabolism (80). Ethnicity also intersects with genetics in dietary choices: a study delved into lactase persistence (LP) genotype's impact on dairy consumption across different ethnic backgrounds. LP individuals exhibited varied dairy preferences, with ethnic background and dietary assessment methods influencing the predictive value of the rs4988235 SNP for dairy intake (81). The potential for personalized dietary recommendations based on genome-wide single nucleotide polymorphism (SNP) data was reviewed by Mullins et al. This article highlighted the intricate gene-diet interactions and molecular phenotypes that inform nutritional recommendations. It emphasized complexities related to genetic architecture, pleiotropy, and epigenetics, providing insight into how genotypic information could guide dietary choices (16). The genetic influence on lactase persistence (LP) or non-persistence (LNP) was also explored in Chilean patients. The LCT C>T−13910 (rs4988235) SNP correlated with lactose intolerance symptoms, showing prevalence variations among Hispanic and Amerindian populations (82).

Alcohol metabolism is another domain intertwined with genetics. Variants of genes encoding enzymes like alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) impact alcohol consumption and the risk of alcoholism. Certain alleles offer protective effects due to altered enzyme kinetics. Noncoding variants in these genes also contribute to alcohol metabolism and alcoholism risk (83). Another study on Black college drinkers found that, while ADH1B3 wasn't directly linked to drinking motives or behaviors, it interacted with motives such as social and coping, influencing drinking quantity and frequency (84). Genetic markers influence alcohol dependence risk. Japanese men and women's alcohol dependence risk was assessed based on alcohol flushing, ALDH2, and ADH1B genotypes: combining flushing and genotypes independently increased alcohol dependence risk (85). ALDH2-rs671 and ADH1B-rs1229984 genotypes influence alcohol-related cancer risk, particularly in the upper aerodigestive tract among East Asian men. ALDH2-rs671 AG genotype exacerbates risks associated with higher alcohol consumption for head and neck, esophageal, and lung cancers (86). An association between aldehyde dehydrogenase 2 (ALDH2) rs671 G>A polymorphism and increased ischemic stroke risk was revealed in a meta-analysis of Chinese subjects. The study emphasized the potential implications of genetic variations in stroke susceptibility (87).

Histamine, a key player in asthma's pathophysiology, has garnered attention for its role in bronchoconstriction and related responses. Investigating the interplay between histamine metabolism and genetic variations in the histamine pathway, a study delved into children with asthma. This research identified associations between genetic variants in ABP1, HNMT, and HRH4 and histamine response parameters, particularly among African American children (88). Central to histamine metabolism is histamine N-methyltransferase (HNMT), which regulates histamine levels in human bronchial epithelium. The HNMT gene's C314T polymorphism has been scrutinized for its impact on enzyme activity and bronchoconstriction. A study focused on Caucasian asthmatic patients and controls, revealing a higher prevalence of the C314T polymorphism in asthmatic individuals. This suggests a potential link between histamine metabolism variation driven by genetic factors and asthma's pathogenesis or treatment response (89). Further insights into HNMT's role emerged from an enzyme activity study involving volunteers. The HNMT C314T genetic polymorphism was examined in relation to histamine regulation. Individuals with the heterozygous CT genotype exhibited lower enzyme activity and elevated plasma histamine levels compared to those with the homozygous CC genotype. This highlights the potential impact of genetic variations on histamine metabolism (90). Table 2 displays SNPs involved in food intolerances.

Tyramine Intolerance: Unraveling the Genetics of Dietary Response

In the intricate interplay between genetics and nutrition, the phenomenon of tyramine intolerance emerges as

Table 2. SNPs with Food Intolerance

RsID	Gene	Function	Alleles	wt/mt	References
rs4988235	LCT	Production of lactase also in adulthood	A/A	wt/wt	(80-82)
		Reduced production of lactase in adulthood	A/G	wt/mt	
		Stops producing lactase in adulthood	G/G	mt/mt	
rs2066702	ADH1B	Typical	G/G	mt/mt	(16)
		Faster metabolism of alcohol with possible acetaldehyde accumulation (more common in African populations)	A/G	wt/mt	(15, 83, 91)
		Faster metabolism of alcohol with possible acetaldehyde accumulation (more common in African populations)	A/A	wt/wt	
rs671	ALDH2	Slower clearance of acetaldehyde; alcohol flush reaction; decrease in alcohol consumption	A/A	mt/mt	(83,85,86)
		Alcohol flush reaction; slower clearance of acetaldehyde	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs61816761	FLG	Increased risk of allergies and nickel sensitivity. 5-fold increased risk of peanut allergy. Increased risk of grass pollen allergy	A/A		(87,88)
		Increased risk of allergies and nickel sensitivity. 5-fold increased risk of peanut allergy. Increased risk of grass pollen allergy	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs11558538	HNMT	Reduced histamine degradation	T/T	mt/mt	(89,90)
		Somewhat reduced histamine degradation	C/T	wt/mt	
		Typical	C/C	wt/wt	

a significant player (92). Tyramine intolerance refers to the inability of certain individuals to efficiently metabolize tyramine, a compound found in various foods (93). As a naturally occurring compound resulting from amino acid breakdown, tyramine is present in aged, fermented, and processed foods (94). Although most individuals metabolize tyramine without issue (95), those with tyramine intolerance experience adverse reactions due to its accumulation, resulting in symptoms such as headaches and migraines (96, 97). Genetic factors, including polymorphisms in genes associated with tyramine metabolism, can underlie this intolerance (7).

Examining the genetic underpinnings of tyramine intolerance, a study involving healthy individuals found that genetic variations in OCT1, CYP2D6, and MAO-A (monoamine oxidase A) did not significantly influence tyramine's effects (7). However, the study highlighted that MAO-A mainly contributes to tyramine metabolism, and while blood pressure increased significantly with systemic tyramine levels, the studied genetic factors did not strongly affect this response.

Shifting the focus to a different species (that is, honey bees), genetic factors influencing latent inhibition towards certain odors not linked to food reinforcement were explored (98). Through cross-breeding and genetic mapping, a tyramine receptor gene (Amtyr1) was identified, shedding light on sensory responses and revealing a reinforcement pathway in non-associative learning.

In a study involving Korean children, the relationship between MAOA polymorphisms and ADHD was assessed (99). Significant associations were found, including a protective effect of the rs6323 TT genotype in girls and risk factors associated with specific polymorphisms in boys, emphasizing the potential role of MAOA gene variants in ADHD development.

Focusing on carotid intima-media thickness (IMT) as a marker of atherosclerosis, the relationship between MAOA promoter methylation and IMT was investigated (100). While an initial association between methylation and IMT was observed, further analysis suggested that familial factors shared by twins, including early life environment and MAOA genotype, largely explained this association.

Examining the influence of genetic variants of the MAOA gene in a cohort of Syrian refugees exposed to traumatic events, the interplay between genetic variants, resilience levels, and exposure to traumatic events was explored (101). The study highlighted the MAOA variant's association with psychosocial stress in males, with resilience demonstrating a strong protective effect.

Transitioning to the FMO3 gene, common genetic variants and their impact on FMO3 expression were identified (102). Specific haplotypes showed significant effects on FMO3 promoter activity, suggesting their role in interindividual differences in FMO3 expression.

A study evaluating subjects with odor complaints for trimethylaminuria (TMAU), a disorder caused by impaired TMA metabolism, revealed variants in genes other than FMO3 that may contribute to TMAU (103). Moreover, an investigation in the Sicilian and Sardinian populations found that, while certain FMO3 gene variants were common, the classical TMAU phenotype of strong odor did not always result from the presence of specific variants (104).

The role of Flavin-containing monooxygenase 3 (FMO3) in metabolizing diverse compounds, including drugs, underscores its significance in health and physiology (105). Genetic variations in the FMO3 gene exert a considerable impact on enzyme activity and substrate metabolism, with potential clinical implications in gastrointestinal disorders and drug responses. Shimizu et al. delved into the FMO3 genotype-trimethylamine conversion relationship within a large Japanese cohort with trimethylaminuria. Their findings highlighted the association of severe cases with loss-of-function mutations in FMO3, while moderate and mild cases suggested the involvement of additional factors (106).

Hepatic FMO3, a pivotal player in metabolizing various compounds, is intricately influenced by age, gender, and genetic factors, thereby affecting xenobiotic and endogenous molecule metabolism (107). The genetic variants within the FMO3 gene have direct consequences, as evident in trimethylaminuria cases, where reduced trimethylamine metabolism due to these variants contributes to severe manifestations. Identification of novel mutations, such as V187A/E158K/E308G and K415 frame shift, further elucidates their impact on enzyme activity and disease severity (108). In a study involving healthy subjects and individuals with cardiovascular disease, common polymorphisms in the FMO3 gene (E158K, V257M, E308G) do not appear to be associated with elevated blood pressure or essential hypertension (109).

While FMO3 influences metabolism, other genetic factors play a role in various conditions. In a Pakistani subpopulation exposed to agricultural chemicals, a case-control study highlighted the association between the CYP2D64 allele and sporadic Parkinson's disease (PD), suggesting heightened risk, particularly in toxin-exposed environments. This variant was also linked to an earlier PD onset and a tremor-dominant phenotype (110).

Pharmacogenomics comes into play with CYP2D6 polymorphisms affecting drug treatment outcomes. Puangpetch et al. emphasized the influence of CYP2D6 polymorphisms on risperidone treatment, with poor metabolizers experiencing more severe adverse events. This insight offers the potential for genetic-based dosing recommendations and underscores the need for genotyping and therapeutic drug monitoring (111). Advancements in precision dosing were explored by Wollmann et al., who introduced solanidine metabolism as a biomarker for predicting the CYP2D6 poor metabolizer phenotype. This approach presents a promising avenue for individualized treatment strategies in clinical practice (112). Chan et al. investigated CYP2D6 genetic variations in a Hong Kong cohort, uncovering a high frequency of CYP2D6*36*10 tandems and duplications, with functional alleles being a minority. This genetic variability underscores the complexity of drug metabolism (113).

Pharmacogenetic implications extended to atorvastatin therapy as Zubiaur et al. found that SLCO1B1 phenotype and CYP3A5*3 variant significantly influenced drug pharmacokinetics. Their proposition for incorporating SLCO1B1 in dosing recommendations emphasizes the potential of pharmacogenetics in optimizing drug therapy (114). The pivotal role of genetic variation in drug metabolism was reaffirmed by the study of cytochrome P450 enzymes, like CYP2D6, which contribute to determining metabolizer phenotypes.

The frequencies of these alleles vary across populations, highlighting the importance of preemptive genetic testing for personalized medication outcomes (115). In addition, He et al. conducted a comprehensive investigation into CYP2D6 genetic polymorphisms among healthy Uygur individuals, uncovering 62 polymorphisms and hinting at potential variations in enzyme activity (116). Table 3 presents the list of SNPs associated with Tyramine Intolerance.

Genetics and Taste Preferences

The realm of taste preferences, influencing dietary choices and nutritional status, is also intertwined with genetics (17). Genetic polymorphisms can impact an individual's ability to perceive different tastes, leading to varying inclinations towards certain foods (118). Universally, people gravitate towards foods that provide pleasurable sensations, encompassing both flavor and texture. Genetic diversity contributes to variations in perceiving and relishing taste qualities like sweetness and bitterness, where factors such as early experiences, learning, genetics, and perceptual nuances contribute to these differences (119). In examining the role of genetics in food preferences, a study delved into the taste and food preferences of Hungarian and Roma populations, exploring potential genetic associations (120). Initial connections were observed between specific genetic polymorphisms and taste/food preferences, although significance waned after multiple test correction. Nonetheless, promising ethnicity-specific effects emerged, suggesting that genetics could indeed influence food preferences, hinting at the need for personalized interventions to modify diets.

Variations in taste perception extend their impact to eating habits and obesity risk, as revealed by another study that investigated associations between genetic variants in taste receptors and taste perception (12). This exploration unveiled substantial links between particular genetic variants and taste function, offering potential implications for personalized nutrition recommendations and dietary patterns. Further investigations into genetic variations in taste receptors explored the relationship between the sour taste receptor SNP KCNJ2-rs236514 and nutrient intake, metabolic health markers, and overall health outcomes (121). Associations with lower fat and specific nutrient intakes, along with metabolic health markers, underscore the potential influence of genetics on health outcomes through taste perception. A study involving young Swedish adults aimed to understand genetic factors' impact on taste and food intake-related ge-

Table 3. SNPs for Tyramine Intolerance

RsID	Gene	Function	Alleles	wt/mt	References
rs6323	MAOA	Typical	G/G	wt/wt	(7,99-101)
		Somewhat reduced enzyme activity	G/T	wt/mt	
		Reduced enzyme activity; possibly decreased tyramine metabolism	T/T	mt/mt	
rs1736557	FMO3	Decreased enzyme function	A/A	mt/mt	(102-104)
		Decreased enzyme function	A/G	wt/mt	
		Typical	G/G	wt/wt	
rs3832024	FMO3	Decreased enzyme function	-/-	mt/mt	(105,106)
		Decreased enzyme function	-/TG	wt/mt	
		Typical	TG/TG	wt/wt	
rs61753344	FMO3	Decreased enzyme function	T/T	mt/mt	(107-109)
		Decreased enzyme function	G/T	wt/mt	
		Typical	G/G	wt/wt	
rs3892097	CYP2D6	Reduced enzyme function. Poor metabolizer	T/T	mt/mt	(111,117)
		Typical	C/T	wt/mt	
		Extensive metabolizer	C/C	wt/wt	
rs5030655	CYP2D6	Enzyme deletion. Poor metabolizer	-/-	mt/mt	(112,113)
		Intermediate metabolizer	-/A	wt/mt	
		Typical	A/A	wt/wt	
rs28371706	CYP2D6	Typical	G/G	wt/wt	(114-116)
		Carrier of one decreased or non-functioning allele	A/G	wt/mt	
		Possibly decreased or non-functioning	A/A	mt/mt	

nes (122). This exploration identified associations between specific gene polymorphisms and taste thresholds, preferred sweet taste intensities, food preferences, and even caries status. These findings highlight the intricate interplay between genetics, taste perception, dietary choices, and the susceptibility to sugar-dependent dental caries. Further unveiling the connection between genetics and taste preferences, a study in the Lithuanian population elucidated associations between genetic factors and taste preferences for sweetness, bitterness, sourness, and saltiness (123). Additionally, the study introduced a useful questionnaire for evaluating taste preferences qualitatively and expanded on previous genetic research within distinct human populations.

A genome-wide association study involving a massive cohort of UK-Biobank participants conducted by May et al. uncovered the genetic determinants of food liking, revealing a hierarchical map of food-liking dimensions (124). These dimensions included "Highly-palatable," "Acquired," and "Low-caloric," each exhibiting distinct genetic correlations and associations with brain traits and consumption. Genetic variations in taste receptor genes exert a considerable impact on the perception of sweet, umami, and bitter tastes, shaping individual preferences (125). Although genetic factors have been linked to preferences and nutrient intake patterns, environmental influences often take precedence over genetic predispositions in shaping food preferences, as demonstrated by many studies focusing on taste buds. The intriguingly divisive preference for Marmite, a quintessentially polarizing food, was found to possess a genetic basis in a genome-wide association study involving 261 adults (126). Notably, one genome-wide significant SNP and additional markers were identified, underlining the complex interplay between genetics and environmental factors in influencing taste preferences. The profound impact of genetic variations on food preferences extends beyond mere choices, potentially influencing broader health outcomes (127). Table 4 highlights some specific SNPs responsible for variations in bitter taste perception and genetic factors that contribute to an individual's aversion to certain bitter-tasting foods.

Discussion

The intricate world of genetics, marked by subtle variations known as polymorphisms, casts a profound influence on our physiological and nutritional well-being (15). These genetic nuances hold the key to understanding an individual's susceptibility to a spectrum of health conditions and their distinct responses to the foods they consume (14). This exploration into the realm of polymorphisms reveals their far-reaching effects, shedding light on their role in food sensitivities, food allergies, tyramine intolerance, and the intriguing domain of taste and food preferences (58).

At the forefront of this genetic interplay lie food sensitivities and allergies, which are complex physiological reactions to specific dietary components (137). Food sensitivities encompass a diverse array of adverse responses that do not trigger an immediate immune reaction (42): these responses—which may manifest as gastrointestinal discomfort, bloating, fatigue, or headaches—can arise from a multitude of factors, including genetic predisposition (138). In contrast, food allergies incite an immune-mediated response that can lead to severe, potentially life-threatening reactions (42). The intricate dance between SNPs and food sensitivities/allergies unveils a captivating glimpse into the genetic foundation of these intricate phenomena (8). Similarly, the lesser-known territory of tyramine intolerance, characterized by the body's struggle to metabolize tyramine efficiently, exposes yet another facet of genetic impact (7). Tyramine, a naturally occurring compound found in specific foods, accumulates in individuals grappling with this intolerance, giving rise to unwelcome symptoms like migraines and headaches (139). Here, SNPs come into play, influencing the performance of enzymes tasked with tyramine metabolism. These genetic variations could potentially contribute to an individual's predisposition to tyramine intolerance (139).

The synergy between SNPs and food sensitivities/allergies reaches beyond mere genetic variation (140). Specific SNPs are linked to an elevated likelihood of developing particular sensitivities or allergies. Polymorphisms in genes

Table 4. SNPs associated with Taste Perception

RsID	Gene	Function	Alleles	wt/mt	References
rs1726866	TAS2R38	Unable to sense bitter in PROP test; likely to consider wine as sweet; can lead to consume more alcohol	A/A	mt/mt	(128-131)
		Able to taste some bitter	A/G	wt/mt	
		Strongly sense bitter in PROP tests; likely to consider wine as bitter; can lead to consume less alcohol	G/G	wt/wt	
rs10246939	TAS2R38	Probably can't taste some bitter flavors	T/T	mt/mt	(132-134)
		Probably can taste bitter	C/T	wt/mt	
		Can taste bitter in broccoli	C/C	wt/wt	
rs35874116	TAS1R2	Lower probability of drinking wine; if drink wine, likely to drink larger amounts	C/C	mt/mt	(135)
		Lower probability of drinking wine	C/T	wt/mt	(136)
		Typical	T/T	wt/wt	(17)

responsible for breaking down certain dietary components—such as lactase for lactose intolerance or HLA-DQ2/DQ8 for celiac disease—can significantly contribute to these responses (46). As the prevalence of food sensitivities and allergies continues to escalate, comprehending the genetic factors that propel these reactions takes on ever greater significance (7). In the realm of tyramine intolerance, a relatively less-explored territory, genetics leaves its imprint as well (106). Genetic markers that impact the metabolism of tyramine have the potential to determine an individual's susceptibility to this intolerance. Crucial enzymes like monoamine oxidase A (MAO-A) play a central role in the metabolic process of tyramine, and SNPs embedded within the MAO-A gene can introduce variations in the way tyramine is processed (99). This genetic variability can ultimately affect an individual's vulnerability to symptoms associated with tyramine intolerance.

Venturing beyond sensitivities and intolerances, SNPs are also deeply involved in the development of an individual's taste and food preferences (58). Genetic variations exert a great influence on an individual's ability to perceive distinct tastes such as sweetness, bitterness, and umami, thereby shaping their inclinations toward specific foods (120). These genetic distinctions in taste receptor genes can lead to divergent preferences and dietary choices (16). For instance, individuals with heightened sensitivity to bitterness might develop aversions to bitter-tasting foods, exerting an impact on their dietary habits and nutritional patterns (123). Furthermore, the genetic patchwork underlying taste and food preferences is enriched through the exploration of unique genetic signatures that steer responses to specific tastes (124). The striking dichotomy between Marmite enthusiasts and skeptics, as illuminated by a comprehensive genome-wide association study, underscores the intricate interplay between genetic predisposition and environmental influences in shaping taste preferences (126). Genetic variations in taste receptors extend their reach beyond mere dietary choices, potentially exerting broader implications on an individual's overall health outcome.

Conclusions

In the rapidly evolving landscape of nutrigenomics, the interplay between SNPs, food sensitivities, allergies, tyramine intolerance, and taste preferences has come to the forefront. Genetic variations paint a personalized portrait of an individual's dietary responses and propensities, providing valuable insights for precision nutrition. By understanding the genetic underpinnings of these phenomena, we pave the way for interventions that align with genetic predispositions, enhancing health and well-being. As research continues to unveil the intricate connections between genetics and nutrition, the potential for personalized dietary strategies becomes ever more tangible, offering a new era of health management rooted in individual genetic uniqueness.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review *Clin Ter 2023; 174 Suppl. 2 (6):227-229 doi: 10.7417/CT.2023.2491*

In Memory of Professor Derek Pheby

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Abstract

Professor Derek Pheby's passing in November 2022 marked a profound loss for the scientific community. Professor Derek Pheby, a stalwart figure in the fields of autoimmune diseases and bioethics, was known for his dedication to scientific research and patients' support, particularly for those affected by paraneoplastic autoimmune syndromes. Professor Pheby made significant contributions to research, especially about Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS). His leadership of the ME Biobank and scientific coordination of EUROMENE demonstrated his commitment to pushing boundaries and fostering international collaborations. Professor Pheby's scientific work addressed various aspects of ME/CFS, from physician education to patient needs, the development of a post-mortem tissue bank, and effective treatments. Beyond his medical career, Professor Pheby was a crucial member of the Independent Ethics Committee of MAGI, he was a poet, humanitarian, and advocate for child protection. His generosity and boundless spirit left an enduring legacy, fostering innovative research in the pursuit of combating autoimmune diseases. ***Clin Ter 2023; 174 Suppl. 2 (6):227-229 doi: 10.7417/CT.2023.2491***

Key words: *Derek pheby, ethics committee, EUROMENE, MAGI, ME/CFS, haldane award*

It was with great sadness that the Independent Ethics Committee of MAGI learned about the passing of one of its most important pillars, Professor Derek Pheby, who peacefully left this word on November 13, 2022.

Professor Derek Pheby was a beacon of inspiration and a true advocate for those affected by paraneoplastic autoimmune syndromes. His legacy resonates through his unwavering dedication to both research and personal support, reflecting his compassionate spirit and remarkable intellect.

Professor Pheby has been involved in the MAGI bioethics committee since its creation and has given it a great deal of support and commitment, including extensive discussions with other committee members and publication of scientific articles (1-3). Professor Pheby introduced MAGI laboratories to research on rare autoimmune diseases, and specifically to the development of the autoimmune retinitis test. Thanks to his mentorship, MAGI is among the few laboratories in Europe — and to date, the only one in Italy — to have a protocol for testing autoimmune retinitis, and is currently collaborating with a prestigious center in the USA.

Professor Pheby's career began at St Thomas's Hospital in London, where he earned his medical degree, to embark

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on a career that comprised medicine, epidemiology, law, and humanitarianism. He moved to the village of Coxwold, North Yorkshire Moors, where he worked as a general practitioner. His pursuit of knowledge and desire to make a broader impact led him to specialize in cancer epidemiology and public health at the University of Bristol, later becoming the Director of the South West of England Regional Cancer Directory.

In his rich and illustrious career, spanning over three decades, Professor Pheby's dedication to advancing rare autoimmune disease research shined brightly, greatly influencing the research landscape, particularly about Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS) (4-16). His involvement in the ME Biobank and his role as the scientific coordinator of EUROMENE, the European research group on ME/CFS, reflected his commitment to pushing boundaries and fostering collaborations across borders. His work alongside fellow academics revolutionized research methodologies, with epidemiologic studies and collaborative initiatives like EUROMENE forging new paths. His leadership of the Socio-economics working group within Euromene highlighted his commitment to understanding the societal impact of ME/CFS, leaving a lasting legacy in the pursuit of a more informed and compassionate world (11, 12,15). Professor Pheby published more than 84 scientific articles in peer-reviewed journals, and he was awarded 17 research grants as principal investigator. He received several awards, among which the Haldane Award from the Royal Institute of Public Administration in 1983 and the Silver Medal of the European Society for Person-Centred Healthcare in 2017. Apart from being the founder of the EUROMENE, he was also member and chairmen of many scientific groups, such as chairman of ENCR data definitions group (1994-1996) and member of MRC Expert Group in ME/CFS (2008-2011).

Professor Pheby's scientific work encompassed many different aspects of ME/CFS research. Indeed, he focused on physician's knowledge of ME/CFS (4-7), underlying the need for improved medical education about ME/CFS and contributing to a better understanding of the challenges faced by healthcare professionals in diagnosing and treating ME/CFS. Moreover, Professor Pheby addressed the needs of patients with ME/CFS (5-8), highlighting the multifaceted nature of patients' challenges – which encompass medical, social, psychological and practical aspects – and underscoring the importance of a holistic approach to their care. Furthermore, he supported the development of a post-mortem tissue bank for ME/CFS (6-9), outlining potential benefits and challenges, ethical considerations, and logistical requirements. He also reviewed the effective treatments for ME/CFS (10-13), identifying patterns and trends that could inform effective treatments based on the diverse population of ME/CFS patients, and analyzing qualitative and quantitative data to assess the effectiveness of various interventions, also considering the patients' perspectives. Finally, his last published scientific articles were included in a special issue dealing with our current knowledge and treatment of ME/CFS (14,16). In these articles, Professor Pheby highlighted the substantial progress that has been made in recent years, shedding light on the complex nature of ME/CFS, addressing the advances in genetics, immunology and neurology, and outlining guidelines for accurate diagnosis and management of ME/CFS.

Beyond his medical endeavors, his interests were as diverse as they were deep. From poetry to politics, he engaged passionately in endeavors that enriched both the artistic and humanitarian spheres. He recently published his first collected poems in the book "Being and Doing and Other Poems". His generosity knew no bounds, and his extraordinary spirit touched everyone fortunate enough to cross his path. He was kind and generous, and his humanitarian passion led him to help the people around him. Indeed, he was awarded the Haldane Prize for his contributions to medical ethics and professional regulation, and especially for his work on child protection.

The activities mentioned above provide just a glimpse of Professor Pheby's significant engagements. His dynamic international influence and his imperative to transcend borders united individuals, fostering innovative collaborations in the shared mission to combat ME/CFS.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review *Clin Ter 2023; 174 Suppl. 2 (6):230-235 doi: 10.7417/CT.2023.2492*

Human Cloning: Biology, Ethics, and Social Implications

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Abstract

This scholarly article delves into the multifaceted domains of human cloning, encompassing its biological underpinnings, ethical dimensions, and broader societal implications. The exposition commences with a succinct historical and contextual overview of human cloning, segueing into an in-depth exploration of its biological intricacies. Central to this biological scrutiny is a comprehensive analysis of somatic cell nuclear transfer (SCNT) and its assorted iterations. The accomplishments and discoveries in cloning technology, such as successful animal cloning operations and advances in the efficiency and viability of cloned embryos, are reviewed. Future improvements, such as reprogramming procedures and gene editing technology, are also discussed. The discourse extends to ethical quandaries intrinsic to human cloning, entailing an extensive contemplation of values such as human dignity, autonomy, and safety. Furthermore, the ramifications of human cloning on a societal plane are subjected to scrutiny, with a dedicated emphasis on ramifications encompassing personal identity, kinship connections, and the fundamental notion of maternity. Culminating the analysis is a reiteration of the imperative to develop and govern human cloning technology judiciously and conscientiously. Finally, it discusses several ethical and practical issues, such as safety concerns, the possibility of exploitation, and the erosion of human dignity, and emphasizes the significance of carefully considering these issues. ***Clin Ter 2023; 174 Suppl. 2 (6):230-235 doi: 10.7417/CT.2023.2492***

Key words: *Human cloning, biology, ethical considerations, social implications, dignity, safety*

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Introduction

Human cloning is the process of making genetically identical duplicates of humans. It involves replicating a person's genetic material in order to create a new organism with the same genetic composition as the original (1,2). Human cloning has a long history, dating back to the pioneering work of Dr. Ian Wilmut, who successfully cloned the first mammal, Dolly the sheep, in 1996. That remarkable achievement generated intense discussions and raised serious ethical concerns about human cloning (3). Both scientific advances and cultural concerns influence the context of human cloning. Indeed, tremendous progress has been achieved in perfecting cloning procedures and expanding our understanding of their uses throughout the years (4,5).

Because of its profound ethical and social consequences, the subject of human cloning has attracted considerable attention and debate. Individual autonomy, the right to genetic identity, potential health hazards, and the broader influence on society and family relations are all discussed in the context of human cloning (6). It is crucial to highlight that human cloning is a challenging and controversial issue, with legal and ethical restrictions in many nations. The ethical implications, risks, and potential benefits of human cloning are still being researched, debated, and discussed in public (7). Human cloning is currently considered unacceptable around the world. The only international legally binding bioethical document to date, the Oviedo Convention from 1997, was quickly expanded to include an amendment banning human cloning in 1998 (8). Later on, with the advances in human stem cell research and the emergence of the concept of therapeutic cloning, the situation became more complicated at the global level. Today, about a quarter of the world's countries have legislation in place banning the cloning of human beings for reproductive purposes. Despite considerable efforts at the UN and UNESCO platforms, the acceptance of a legally binding international law on human cloning has not been achieved (9).

This article intends to investigate the biology, ethics, and societal implications of human cloning by offering a brief summary of the procedure, its historical evolution, and present context. Understanding the foundations and evolution of human cloning is crucial for comprehending the ethical considerations and potential societal impacts associated with this field.

Biology of human cloning

The biology of human cloning seeks to give a comprehensive examination of the scientific principles underlying human cloning procedures. The emphasis is mostly on SCNT, which is one of the most often utilized technologies in human cloning (10). SCNT includes extracting the nucleus from a somatic cell, which contains the donor's genetic material; the somatic cell nucleus is then united with an enucleated egg cell. The genetic material from the somatic cell is fused with the cytoplasm of the egg cell, resulting in a reconstructed egg cell or reconstructed embryo with the same chromosomal genetic information as the original somatic cell donor. This reconstituted egg cell is stimulated to divide and develop further, with the goal of producing an embryo capable of developing into an organism genetically identical to the somatic cell donor (11). By transferring the genetic material from a somatic cell into an enucleated egg cell, SCNT allows for the creation of a cloned embryo with the same genetic makeup as the original donor. This process forms the basis of human cloning and has been utilized in various scientific experiments and research activities (12).

Advancements in cloning technology have led to significant progress in the SCNT process, resulting in improved efficiency, safety, and ethical considerations in human cloning, as shown in Table 1. Notable achievements and variations in the field have contributed to our understanding of cloning techniques (13). One area of advancement is the refinement of nuclear transfer techniques: investigators have

Table 1. Advancements and Variations in Cloning Technology

Advancements/Variations	Description	Implications
Improved Nuclear Transfer Techniques	Refining the transfer process for enhanced efficiency and success rates	Higher success rates and improved outcomes in cloning experiments
Alternative Cell Types for Cloning	Exploration of using stem cells or cells from different body tissues for nuclear transfer	Expanded applications and understanding of different cell types
Induced Pluripotent Stem Cells (iPSCs)	Reprogramming adult cells into pluripotent stem cells for cloning	Overcoming ethical concerns and potential for diverse cell sources
Mitochondrial Replacement Therapy	Replacement of defective mitochondria with healthy mitochondria from donors	Prevention of mitochondrial diseases and advancement in assisted reproductive technology
Animal Cloning	Successful cloning of animals, such as Dolly the sheep	Insights into cloning techniques and optimization of procedures
Efficiency and Viability of Cloned Embryos	Improvement in the survival and normal development of cloned embryos	More reliable and consistent outcomes in cloning experiments
Reprogramming Techniques	Understanding the molecular mechanisms of somatic cell reprogramming	Enhanced efficiency and quality of cloned embryos
Nuclear Transfer Variations	Exploration of variations like pronuclear transfer or spindle transfer	Potential advantages in efficiency and prevention of genetic disorders

optimized the transfer process, to minimize genetic material damage and ensure proper integration of the transferred nucleus. These improvements have enhanced the success rates and reliability of cloning (14). Exploration of alternative cell types for cloning has also expanded the potential of this technology. For example, stem cells and cells derived from different body tissues have been investigated as viable options for nuclear transfer, broadening the potential applications and providing insights into the capabilities of diverse cell types (15).

Reprogramming techniques have played a crucial role in successful cloning. Understanding the molecular mechanisms involved in reprogramming somatic cell nuclei has led to improved techniques for converting differentiated cells into a pluripotent state (16). These advancements, such as using transcription factors or epigenetic modifications, have enhanced the efficiency and quality of cloned embryos. Variations in nuclear transfer techniques have also been explored (17), such as pronuclear transfer, where nuclei of two fertilized embryos are swapped, and spindle transfer, involving nuclear DNA transfer into an enucleated egg cell with preserved mitochondria, which have demonstrated potential advantages in terms of efficiency and prevention of genetic disorders (18). These advancements in cloning technology have deepened our understanding of the process, improved the efficiency and viability of cloned embryos, and addressed ethical concerns. Ongoing research and breakthroughs in the field continue to pave the way for responsible and advanced applications of human cloning (19).

Ethical Considerations

Ethical considerations concerning human cloning involve a range of complex issues. Some key ethical dimensions of human cloning include (20):

Human Dignity: Human cloning raises concerns about the dignity and uniqueness of individual human beings. It challenges the notion that each person is a distinct individual, with inherent worth and rights. Ethical discussions often center on whether cloning undermines or respects human dignity (21).

Autonomy and Consent: Respect for individual autonomy and the principle of informed consent are important ethical principles. Questions arise regarding the right to make informed choices about one's genetic information and the use of that information for cloning purposes (22).

Safety and Well-being: The potential health risks and overall well-being of cloned individuals are significant ethical considerations. There may be concerns about long-term health effects, increased susceptibility to genetic disorders, and potential psychosocial impacts on cloned individuals (23).

Principle of Reciprocity: Balance and fairness is also relevant in the ethical discussion of human cloning. Reciprocity suggests that the benefits and burdens of cloning technology should be distributed equitably among individuals and society (24). This principle calls for careful consideration of the potential social, economic, and psychological impacts of human cloning, and ensuring that, if cloning is approved, any benefits derived from it are shared in a way that promotes equity (25).

Analyzing cause-and-effect correlations is critical in ethical debates about human cloning. The potential effects of cloning on people, families, and society must be considered. Examining the psychological well-being of cloned individuals, the impact on family relationships, the possibility of identity issues, and broader societal consequences, such as the distortion of family structures or the erosion of genetic variety, are examples of ethical inquiries (26).

Overall, the ethical dimensions of human cloning encompass concepts of human dignity, autonomy, safety, fairness, and the potential consequences on individuals and society. Ethical deliberations aim to strike a balance between scientific progress and responsible application, considering the principles of reciprocity and cause-and-effect to ensure the well-being and rights of individuals while upholding broader societal values (27, 28).

Social implications

The social implications of human cloning encompass a wide range of concerns and considerations that extend beyond the individual level. Here are some key aspects of the social implications of human cloning (29):

Personal Identity and Uniqueness: Individuals created through human cloning could replicate the characteristics of their donors to the extent of compromising their uniqueness. This issue may lead to complex psychological and philosophical debates about individuality and personal identity, which should also consider that one's identity is shaped by a combination of genetic inheritance and individual experiences (30).

Family Dynamics and Relationships: The presence of cloned individuals may disrupt traditional concepts of family structure and kinship. Questions arise regarding how cloned individuals relate to their genetic donors, their relationship with their cloned siblings, and their place within the family unit (31).

Concepts of Parenthood: In terms of social concerns, human cloning challenges conventional concepts of parenthood, blurring the lines between biological and social parenting. Cloning raises questions about the roles, responsibilities, and rights of genetic donors, surrogate mothers, and individuals involved in the upbringing of cloned individuals, which have significant social implications (32,33).

The availability of human cloning as a reproductive option raises social concerns about its impact on traditional reproductive choices (34). Debates center around whether cloning could overshadow or replace other assisted reproductive technologies, such as in vitro fertilization (IVF), and the potential consequences of limiting the range of reproductive options available to individuals and couples. Furthermore, the accessibility and affordability of cloning technology may create social and economic inequalities (35).

Considering all these social implications is essential to ensure a comprehensive understanding of the potential consequences and challenges associated with human cloning. Ethical considerations, public discourse, and societal engagement are crucial in navigating the complex social landscape surrounding cloning technology, fostering a balanced approach that addresses concerns while promoting

the well-being and rights of individuals and society as a whole (36).

Cautions and Related Problems

Exercising caution is highly important in the field of human cloning, due to various ethical and practical challenges associated with this technology. It is crucial to consider the following cautions and related problems (37):

- Safety Concerns: The safety of cloned individuals is a primary concern. Despite advancements in cloning techniques, there are still potential risks associated with the development and health of cloned embryos and resulting individuals. Further research and long-term studies are necessary to fully understand and mitigate these safety concerns (38).
- Exploitation and Commercialization: Human cloning raises concerns about its potential use for exploitation and the commercialization of human life. If cloning becomes a commercial enterprise, there is a risk of treating human life as a commodity, compromising ethical principles and human dignity. Strict regulations are necessary to prevent the unethical exploitation of cloning technologies (39-41).
- Psychological and Social Impacts: Cloning may have psychological and social impacts on cloned individuals, families, and society. Cloned individuals may face identity issues and questions about their place in society. The potential social challenges related to their unique genetic status should be carefully considered (42).
- Ethical Boundaries: Human cloning tests the boundaries of ethical principles, societal norms, and cultural values. Engaging in comprehensive ethical discussions and seeking input from diverse perspectives is essential to determine the acceptable limits and responsible use of cloning technologies (43,44).

Approaches for the development and regulation of human cloning technologies

By emphasizing cautions and related problems, it is crucial to promote thoughtful and responsible approaches for the development and regulation of human cloning technologies. Implementing robust ethical guidelines, rigorous scientific research, and comprehensive public engagement can help navigate the complexities and challenges of human cloning, ensuring that advancements in this field align with principles of safety, dignity, and individuals' and society's well-being (45,46). Specifically, some key aspects to develop regulations for human cloning technologies are:

- Comprehensive Ethical Framework: Establish a comprehensive ethical framework that addresses the potential risks, safeguards human dignity, and promotes the well-being of individuals. This framework should be developed through multi-stakeholder engagement, including input from experts in bioethics, policymakers, scientists, and representatives from diverse societal groups (47).
- Robust Scientific Research: Prioritize ongoing scientific research to fully understand the safety, long-term effects, and potential consequences of human cloning. Rigorous studies, including preclinical and clinical trials, should be conducted to ensure that cloning technologies are based on sound scientific evidence and have minimal risks to the health and well-being of cloned individuals (48).
- Transparent Regulation: Establish open regulatory frameworks to oversee the development and use of cloning technologies. These policies should address concerns about safety, assure informed consent, avoid exploitation, and protect against unethical behavior. Regular monitoring and evaluation should be in place to adapt regulations as scientific knowledge and societal values evolve (49, 50).
- Public Engagement and Dialogue: Promote open and inclusive public engagement to inform the development and regulation of cloning technologies. Encourage public discourse, education, and awareness campaigns to ensure that the broader society has a voice in shaping the ethical boundaries, social acceptability, and potential applications of cloning. Public input should be considered in policy-making processes (51).
- International Collaboration and Harmonization: Promote international collaboration and cooperation to address the global implications of human cloning. Encourage dialogue among different countries and regulatory bodies to establish harmonized guidelines and standards. International cooperation can help prevent the emergence of unethical practices, facilitate shared research efforts, and ensure consistent ethical considerations across borders (52).

By implementing such a thoughtful and responsible approach, the development and regulation of human cloning technologies can balance scientific progress with ethical principles, ensuring the safety, dignity, and well-being of individuals while fostering public trust and social acceptance.

Conclusions

Human cloning is a complex and multidimensional field with significant scientific, ethical, and social implications. The advancements in cloning technology, including improvements in nuclear transfer techniques, alternative cell types, and scientific progress in animal cloning, have expanded our understanding of the cloning process and its potential applications. However, caution must be exercised in the development and regulation of human cloning technologies. Ethical, legal and social considerations, such as human dignity, autonomy, and safety, should be paramount in guiding the responsible use of cloning technology.

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Conflicts of interest statement

Authors declare no conflict of interest.

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X-linked genodermatoses from diagnosis to tailored therapy

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Abstract

Background. Genodermatoses are rare heterogeneous genetic skin diseases with multiorgan involvement. They severely impair an individual's well-being and can also lead to early death.

Methods. During the progress of this review, we have implemented a targeted research approach, diligently choosing the most relevant and exemplary articles within the subject matter. Our method entailed a systematic exploration of the scientific literature to ensure a comprehensive and accurate compilation of the available sources.

Results. Among genodermatoses, X-linked ones are of particular importance and should always be considered when pediatric males are affected. Regardless of other syndromic forms without prevalence of skin symptoms, X-linked genodermatoses can be classified in three main groups: keratinization defects, pigmentation defects, and inflammatory skin diseases. Typical examples are dyskeratosis congenita, keratosis follicularis spinulosa decalvans, hypohidrotic ectodermal dysplasia, chondrodysplasia punctata, hypohidrotic ectodermal dysplasia, incontinentia pigmenti, chronic granulomatous disease, CHILD syndrome and ichthyosis. In this field, genetic diagnosis of the specific disease is important, also considering that numerous clinical trials of orphan drugs and genetic therapies are being proposed for these rare genetic diseases.

Conclusions. Thus, this chapter starts from clinical to molecular testing and ends with a review of all clinical trials on orphan drugs and gene therapy for genodermatoses. ***Clin Ter 2023; 174 Suppl. 2 (6):236-242 doi: 10.7417/CT.2023.2493***

Key words: *genodermatosis, X-linked, hyperkeratosis, gene therapy, genetic diagnosis, tailored therapy*

Introduction

Genodermatoses

Genodermatoses are a group of inherited skin disorders that are caused by genetic defects like mutations or deletions of one or multiple genes (1). Genodermatoses typically affect the skin, hair, nails and sometimes other organs of the body. Due to a wide amount of genetic variation, multiple types of genodermatoses exist like ichthyosis or xeroderma pigmentosum. Symptoms therefore vary widely depending on the specific condition. Common symptoms include dry, scaly, or thickened skin; blisters or sores; and abnormal hair or nails. In some cases, genodermatoses can also cause problems with the eyes, teeth or internal organs (1).

Genodermatoses can occur in people of all ages and races, but some conditions are more common in a certain population. For example, ichthyosis is more common in people of African descent while xeroderma pigmentosa is more common in people of Japanese descent (1).

X-linked genodermatoses

X-linked genodermatoses are a group of genodermatoses disorders where the genetic causal factors are located on the X chromosome (2). These disorders are called X-linked because they are caused by mutations on the X Chromosome, one of the two sex chromosomes. Since females have two X chromosomes and males have one X and one Y chromosomes, the way that X-linked disorders are inherited and expressed can be different between males and females. Typically, symptoms will be present in males whilst females who carry the mutation on one of their X chromosomes may be affected to a lesser degree or not at all, as the second X chromosome can compensate for the mutation (1). X-linked genodermatoses can be further classified in three main groups:

Keratinization defects

In this group, the genodermatoses in caused by abnormal development or metabolism of the protein keratin in the skin cells. Keratin is a protein that gives the skin strength and integrity and it is important for the formation of the skin's barrier function. When there is a defect in the process of keratinization, the skin can become dry, scaly and prone to infection. Treatment often involve creams and ointments on the skin (3).

Pigmentation defects

Pigmentation defects are caused by abnormal development or metabolism of the pigment melanin in the skin cells. Melanin is the pigment that gives color to the skin, hair and eyes and it is essential for the protection of the skin from harmful effects of ultraviolet (UV) radiation. When there is

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a defect in the process of pigmentation, the skin can become lighter or darker than normal, or have uneven patches of color. This can particularly be the case in women due to mosaic X-chromosome inactivation (4).

Inflammatory skin diseases

In this group, the skin disease is caused by an inflammation reaction of the skin. This can result in redness, itching and scaling leading to eczema, psoriasis or acne. However, these conditions are also dependent of environmental factors (5).

Examples of X-Linked genodermatoses

Dyskeratosis congenita

Dyskeratosis congenita, also known as Zinsser-Engman-Cole syndrome, is characterized by skin changes, nail dystrophy, and leukoplakia (white patches in the mouth). The disease can also be accompanied by more serious consequences like bone marrow failure, leading to insufficient blood cell production, and cancer. Dyskeratosis congenita can be inherited and is caused by mutations in genes involved in telomere maintenance that lead to telomere shortening. The disease is diagnosed based on symptoms and confirmed by genetic testing. 19 different genes are found to be involved in dyskeratosis congenita with 1 out of 5 of the pathogenic mutations found in *DKC1*, a gene encoding for dyskerin (6).

Keratosis follicularis Spinulosa decalvans

Spinulosa decalvans is a genodermatosis affecting the follicles of the hair and is characterized by scarring alopecia on the scalp, eyebrows and axillae. Being X-linked, it is predominantly present in men and rarely in women (7). The disorder is caused by mutations in the *MBTPS2* gene encoding an intramembrane zinc metalloprotease involved in sterol control of transcription and the ER stress response called the membrane bound transcription factor peptidase, site 2 (or S2P) (8).

Hypohidrotic ectodermal dysplasia

Hyophidrotic ectodermal dysplasia is an ectodermal disorder leading to abnormal development of skin, hair, nails, teeth and sweat glands before birth (9). Patients have fewer or non-functional sweat glands leading to a reduced ability to sweat (hypohidrosis). This can result is serious problems with body temperature control. Patients will have little to no hair and absent or malformed teeth. The disorder is also associated with distinctive facial features like thin lips, flattened nose bridge and a prominent forehead. The X-linked form is caused by genetic mutations in the *EDA* gene (10) which is responsible for crosstalk between the ectoderm and mesoderm during development.

Chondrodysplasia punctata

There are 4 types of chondrodysplasia punctata of which 2 are X-linked. The Gondradi-Hunermann type is caused by mutations in the EBP gene (11). Patients have a short stature, low nasal bridge and skin lesions. The Rhizomelic form is caused by mutations in the PEX7 gene and is characterized by short extremities, cataracts, ichthyosis and nasal hypoplasia. Patients will rarely survive past infancy. The X-linked recessive type is caused by mutations in the *ANOS1* gene whilst the X-linked dominant (also known as Happle syndrome) form is caused by defects in the *EBP* gene. X-linked chondrodysplasia punctata is a developmental disorder of bone and cartilage. The disease presents as spots in cartilage and near the end of bones on X-Rays. It mostly occurs in the bones of the toes, fingers and ankles. In addition, patients will have short stature and short tips of fingers and toes. Furthermore, the patients will have a flattened nose with a flattened nasal bridge and crescent shaped nostrils (11).

Incontinetia pigmenti

Incontinentia pigmenti is a condition that mainly effects the skin. During infancy, patients will have a blistering rash followed by wart-like skin growth (12). During childhood, the wart growths will become grey-brown patches that eventually evolve in lighter patches during adulthood. In addition to skin symptoms, patients will experience hair loss, small or missing teeth, fingernails and toenails abnormalities and eye abnormalities (13). The disorder is caused by mutations in the *IKBKG* gene encoding the NEMO protein, which protects the cell from TNF-alpha induced apoptosis (12).

Chronic granulomatous disease

Chronic granulomatous disease (CGD) is an immunodeficiency disorder in which white bloods cells are unable to kill certain bacteria and fungi, making patients with CGD at a high risk for frequent bacterial and fungi infections leading to recurrent infections (14). In addition, patients may suffer from abscesses in their liver, spleen bones, lungs and skin as well as granulomas in the bowel and urinary tracts. The disease is caused by defects in the NADPH oxidase complex, an enzyme complex build up from different proteins from multiple genes. Being an X-linked disorder, hemizygous or heterozygous mutations in the p91-phox (*CYBB*) gene can lead to CGD (15,16).

CHILD syndrome

Congenital hemidysplasia with ichthyosiform erythroderma and limb defects, also known as CHILD syndrome, is a dominant X-linked condition leading to ipsilateral symptoms in multiple organs. All patients will have unilateral erythematous skin plaque with a midline demarcation from birth (17). In addition, patients will suffer from musculoskeletal problems like hypoplasia to agenesis, possibly leading to scoliosis. CHILD syndrome is caused by mutations in the *NSDHL* gene encoding the NAD(P)H steroid dehydrogenase-like protein. This protein is responsible for the dehydrogenases of 3beta-hydroxy sterols that is vital for the production of cholesterol (18). This leads to a shortage of cholesterol for proper formation of membranes and myelin around nerve fibers (19).

Ichthyosis

Patients with X-linked ichthyosis are characterized by dark brown scales and dryness of the skin (20). The symptoms are congenital and more often do not improve with age. The disorder results from mutations in the *STS* gene leading to steroid sulfatase deficiency (21). A comprehensive list of X-Linked genodermatoses can be found in Table 1.

Clinical and molecular testing

Genodermatoses, and more specifically X-linked genodermatoses, are a group of heterogeneous skin disorder that are very similar and often difficult to distinguish from each other. Due to the heterogeneity in onset and severity of symptoms, it remains difficult to diagnose the specific type of genodermatoses. In addition, these disorders can each be caused by a mutation in different genes or multiple genes. Successful treatment relies on the correct characterization and identification of the specific disorder to allow for a targeted treatment. Therefore, diagnosing the specific type of genodermatoses is pivotal.

Clinical testing

The clinical expression of genodermatoses is very indistinct with much variants in symptom expression making their genotype-phenotype correlation challenging to determine for the treating physician. In some cases, symptoms can start from birth while in other cases symptoms remain unnoticed until adulthood. All subgroups of genodermatoses were divided into subgroups by to their clinical manifestations (22). These subgroups included: ectodermal disorders; connective tissue disorders; epithelial adhesion disorders; keratinizing disorder; DNA repair disorders; progeroid disorder; pigmentary disorder; tumor predisposition disorder; nail disorder; hair disorder; developmental disorder; vascular disorder; immune disorder; metabolic disorder.

When a patient presents themselves with genodermatosis like symptoms, the first step for correct identification and diagnosis is to perform a detailed clinical examination in order to provide an accurate list of phenotypes. This should include potential phenotypes on the skin, hair, nails and teeth. The second step involves a detailed anamnesis as well as the medical history of a three-generation family pedigree in order to evaluate the mode of inheritance. Finally, it is often needed to perform laboratory testing to find the proper diagnosis (22).

Laboratory testing for genodermatoses includes histopathology, electron microscopy and immunofluorescence. Histopathology can be most helpful in cases such as Darier disease or incontinent pigmenti. Using histopathology, it is possible to visualize classical cellular manifestations or certain types of genodermatoses like apoptotic keratinocytes presenting with dark and fragmented nuclei in Darier disease (22).

Molecular testing

Next to clinical evaluation, molecular testing can provide pivotal information for the diagnosis of genodermatoses. Due to the boom in DNA technologies available to researchers, it was possible to identify genes associated with genodermatoses using genetic linkage analysis and positional cloning (23). Technologies like DNA sequencing and RNA profiling therefore make it possible to easily identify which gene is involved in patients skin disorder and ergo which type of skin disorder the patient has.

Next generation sequencing (NGS) allows for massive parallel sequencing of DNA to simultaneously sequence hundreds of genes (24). Using this technique, researchers can perform a whole exome sequencing (WES) on patient DNA. The whole exome contains all of a patient genes, therefore, WES data can reveal any mutation in any gene. Currently, the diagnostic rates of WES are estimated at 30-60% for all genodermatoses, making it a popular tool for diagnosis of genodermatoses (25). Certain subgroups of genodermatoses like epithelial adhesion disorders and keratinization disorders have an 80-100% average diagnostic rate using DNA sequencing (26).

In other cases, the DNA itself can remain healthy without any mutations while the gene expression levels have changes beyond healthy levels. Without any expression of the gene, a patient will in most cases have the same phenotypes as if the gene was mutated. Screening for levels of RNA can therefore be key in diagnosis genodermatoses. RNA-Sequencing can be used to identify, quantify and compare RNA expression levels in a patient. There are three types of changes to RNA that can be identified using RNA-seq. 1) Detecting changes in expression of certain genes outside of their physiological boundaries. This can be caused due to epigenetic or genetic changes to promoters or enhancers that regulate the gene's expression (27). 2) Detecting changes in splicing patters that lead to an aberrantly spliced gene due to changes in intronic

Table 1. X-Linked genodermatoses, OMIM number, causative gene, OMIM number.

X-Linked Disease	OMIM	Gene	OMIM
Dyskeratosis congenita	305000	DKC1	300126
Spinulosa decalvans	308800	MBTPS2	300294
Hypohidrotic ectodermal dysplasia	305100	EDA	300451
Chondrodysplasia punctata	302950	ANOS1 EBP	300836 300205
Incontinetia pigmenti	308300	IKBKG	300248
Chronic granulomatous disease	306400	CYBB	300481
CHILD syndrome	308050	NSDHL	300275
Ichthyosis	308100	STS	300747

DNA segments (28). 3) Detecting changes in gene expression due to an imbalance in allele-specific expression (29).

In addition to evaluating the gene expression levels of the patient, RNA-seq can be used to detect foreign gene expression. This way, researchers can detect the presence of viruses that can causes viral dermatoses (30).

Clinical trials on orphan drugs and gene therapy

The advent of new diagnostic tools like DNA and RNA sequencing has led to an increase in genodermatoses diagnoses. In many cases, these skin disorders markedly affect the patients' quality of life and can lead to an increase in cancer risk (31). Currently, 1/2000 people have a type of genodermatoses. For these people, treatment is limited to skin and wound care using topical oils and creams, surgery or pain and itch treatment (32). In addition, patients can learn how to manage their symptoms and how to avoid certain triggers that might evoke symptoms. As these treatments are needed life-long, it can be economically challenging for patients to adhere to the full treatment plan (32). Due to the advances of scientific research over the past 20 years, researchers are starting to unravel the molecular and genetic background of genodermatoses. Unraveling the underlying pathophysiology can lead to novel and more targeted and specific methods for the treatment of genodermatoses. There are 3 different types of approaches to treat genodermatoses.

Interfering in the pathophysiological pathways

In some genodermatoses, a certain pathway is over- or under-activated, leading to an aberrant cellular environment. For many cellular pathways, there already exist drugs to increase or decrease the activity of the pathway. Using these drugs, doctors can choose to target the aberrantly regulated pathway. For example, in neurofibromatosis 1 (NF1), the mitogen-activated protein kinase (MAPK) is overactive leading to the NF1 symptoms. Decreasing the MAPK pathway using MAPK inhibitors is therefore an attractive treatment option (33).

Targeting the inflammatory pathways

In many genodermatoses, the symptoms are in part caused by an exaggerated inflammatory reaction. These symptoms are most often treated by targeting hyperkeratosis and using anti-inflammatory creams based on corticosteroids (34). In recent years, doctors are opting for therapies targeting the inflammatory process like TNF-α inhibitors, antibodies or cytokines (35). These treatments were proven to successfully reduce inflammation levels, alleviating symptoms.

Restoration of the underlying gene or protein defect

In some cases, genodermatoses is caused by a DNA defect like mutations, deletions or insertions that lead to a complete loss of that gene. As genes are transcribed into RNA, which later translates into proteins, loss of a gene due to mutations will downstream lead to a loss of the protein encoded by the gene. The pathophysiology of the specific genodermatoses will in that case be caused by the loss of the protein from the cellular environment. Restoring the gene or replenishing the protein will in that case result in a restoration of the defects caused by the absence of the protein. This can be achieved in multiple ways. First, protein therapy aims to replace the lost protein by providing the protein to the cells. This protein can be made in a lab and administered to the cells by in injections or drugs. For example, researchers were able to cure a mice model for dystrophic EB, a genodermatosis due to missing type 7 collagen (C7), by providing C7 by injection. The collagen is able to reach the skin and for anchoring fibrils at the dermo-epidermal junction, thereby performing their natural function and restoring the disorder (36). Protein therapy can often be challenging as the protein needs to be supplied in physiological levels as too little or too much protein could disrupt the physiological balance. In addition, the protein often needs to be administered at specific times, for example, some conditions are caused by defects during embryonic development. In these cases, suppling the protein will only have a positive effect during development. Secondly, Cell therapy can be used to restore the loss of a protein by administering health cells that can make this protein. For example, loss of collagen type 7, which made by fibroblasts, can be restored by providing allogeneic fibroblasts of a health donor. These cells can settle within the host patient and naturally produce C7 and thereby restore the disorder (37). Lastly, Gene therapy is a novel option to replace or restore the defect gene to restore the disorder (38). Given that all genodermatoses have a genetic defect or predisposition, principally, all genodermatoses could benefit from gene therapy. In Gene therapy, doctors can provide DNA, RNA or gene editing tools inside the cells of a patient and thereby restore the gene defect. These gene therapies can be distributed inside a patient by used non-integrating viral vectors or lipid or polymeric nanoparticles. However, there are still many hurdles to overcome for gene therapy to be safe and effective. Different kind of gene therapies are currently being tested and used. 1) Gene insertion: with gene insertion, researchers and doctors will supply a healthy copy of the mutated gene to the cells. This healthy copy can live outside the genome and replace the defective, mutated, gene (39). 2) Gene editing: instead of replacing the defective gene, gene editing aims to restore the naturally present gene by modifying or restoring the gene in the genome itself. This can be achieved by a variety of molecular tools like zinc finger nucleases (ZNFs), transcription-activator like effector nucleases (TALEN) and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) (40). These tools are able to make precise cuts in the genome that can be repaired by supplying a repair template via homology directed repair. This repair template contains the healthy/normal DNA of the mutated DNA causing the disorder. 3) RNA-based therapy: contrary to the previous techniques that aim to restore the defective DNA, RNA-based therapy aims to provide healthy levels of RNA that can naturally be transcribed into protein to restore to protein deficiency (41). In other cases, different types of RNA can be exogenously suppled that can interfere with splicing, transcription or transport of other genes thereby regulating overall gene expression in the cell. RNA-based techniques included: small interfering RNAs (si-RNA), antisense oligonucleotides (AONs), spliceome-mediated RNA trans-splicing (SMaRT) and micro RNAs (miRNAs) (41).

Genetic skin disease itching

Many of the genetic (X-linked) skin diseases involve some form of itching that has a negative impact on the patient's quality of life. A new clinical trial at the children's hospital of Chicago aims to evaluate the treatment of dupilumab on improving itch for patients with genetic inflammatory skin disorders (NCT05649098). The study has enrolled 30 participants and has started on February 1st, 2023. The study is scheduled to complete on February 1st, 2026.

Dyskeratosis congenita

Treatment for dyskeratosis congenita often involves stem cell therapy. To prepare the bone marrow for stem cell transplantation, patients receive high dose of pre-transplantation radiation and chemotherapy. This can often be intolerable for some patients, lead to side effects and result in slow recovery of blood counts. A new study evaluated if lower dose chemotherapy and radiation followed by stem cell transplantation is effective for patients with dyskeratosis congenita (NCT00455312). Researchers created a new nonmyeloablative conditioning regimen and tested it on 6 patients (42). Five of the six patients successfully engrafted the donor cells. After 26.5 months, four out of the six patients are alive. Thereby they conclude that the novel nonmyeloablative conditioning regimen is successful in prepare patients to receive stem cell therapy and can lead to successful engraftment of the stem cells without cause adverse events as occurs in the normal conditioning regimen (42).

Hypohidrotic ectodermal dysplasia

Hypohidrotic ectodermal dysplasia affects several ectodermal structures which leads to an impaired sweat glands, teeth development, and meibomian glands. This disorder is caused by the absence of ectodysplasin A (EDA) during development. Therefore, recent therapeutic approaches aim to replace this protein with synthetic proteins. In 2018, Schneider et al. administered fusion protein Fc-EDA prenatally to the amniotic cavities of three fetuses from two different pregnancies. In both cases, sweat glands, meibomian glands and teeth developed normally compared to (43). Currently, a new study aims to administer EDA immediately after birth to restore normal development (NCT01775462).

Another study aims to evaluate the efficacy of ER004, a first-in-class protein replacement molecule designed to perform a high-affinity binding to the endogenous EDA1 receptor (NCT04980638). When bound to the receptor, ER004 activates the EDA/NF_KB pathway to trigger normal development. This protein replacement clinical trial aims to evaluate the efficacy and safety of intra-amniotic ER004 administrations as a treatment option for patients with X-linked hypohidrotic ectodermal dysplasia.

Chronic granulomatous disease

X-linked chronic granulomatous disease (X-CGD) is caused by a defect in phagocytic cells ability to produce reactive oxygen species due to a mutation in the gp91phox subunit of NADPH oxidase. Currently, the treatment option of choice Is an allogenic hematopoietic stem cell transplantation to restore the ability of the body to naturally produce phagocytic cells. However, this treatment is subject to immunological rejection from the host. Therefore, a new clinical trial at the University Hospital of Frankfurt aims to treat with gene-corrected autologous CD34+ cells by using a SIN gammaretroviral vector for an *ex-vivo* gene therapy (NCT01906541). 5 participants were enrolled in the trial. From these patients, CD34+ cells will be extracted and cultured. These cells carry a mutation in the gp91phox gene that will be replaced by ex-vivo gene therapy before returning the corrected cells back inside the patient's body. As these cells are originating from the patient, they will likely not be rejected when reinserting. Similarly, a new study at the Children Hospital of Paris, France, aims to restore patients CD34+ cells with lentiviral transduction of the XCGD gene (NCT02757911). 3 participants are enrolled in this study which is set to end on June 2024.

These clinical trials have a large potential for success. Previously, Donald B Kohn et al. (2020) reported on the result of 2 clinical trials (NCT02234934 / NCT01855685) in which patients with hematopoietic stem cells receives ex vivo lentiviral gene therapy (44). After 12 months, 6 of the 7 patients showed 16 to 46% oxidase-positive neutrophils. These patients had no new X-CDG related infections and have been able to stop x-CDG antibiotics (44).

CHILD syndrome

Patients with CHILD syndrome suffer from congenital ichthyosis, erythroderma, and hyperkeratosis. The treatment of these conditions often involves systemic retinoids which may cause dose dependent adverse events. Recently, researchers made a novel topical isotretinoin ointment formulation to treat congenital ichthyosis (45). The formulation termed PAT-001 is patented for this use. In total, 19 patients enrolled in a clinical trial to test the safety and efficacy of PAT-001. In total, seven patients discontinued the trial and in total 28 adverse events occurred over 14 patients. However, scaling was significantly reduced in the treated group. More testing is required to make a proper cost/benefit analysis to outweigh the adverse events against the improved scaling.

Ichthyosis

X-linked ichthyosis is mainly characterized by hyperkeratosis and widespread scaling treated with oils and ointments or systemic retinoids. A new randomized, double-blind study evaluated the effect of TMB-001, a novel topical isotretinoin ointment on hyperkeratosis and scaling (NCT04154293) (46). In total, 33 patients enrolled in the study of which 11 received a 0.05% and 10 a 0.1% TMB-001 ointment treatment. Researchers found that TMB-001 demonstrated significant improvements to the patients' skin after 12 weeks of treatment. Furthermore, TMB-001 0.05% was more effective than 0.1%.

Another double blind, randomized control trial evaluated the effect of oral vitamin D on patients with congenital ichthyosis compared to a control group receiving acitretin treatment (47). Eleven patients received 2000 IU vitamin D a day for 24 weeks. Patients treated with oral vitamin D showed a significant decrease in the visual index for ichthyosis severity and area severity index after 12 weeks of treatment. However, this effect was not visual after 24 weeks (47).

Conclusion

Genetic testing is widely used in the diagnosis of X-linked genodermatoses and should always be considered after clinical diagnosis, especially when pediatric males are affected. Indeed, identifying the underlying genetic basis of these diseases will allow the development of gene therapies targeting the specific mutated genes. Considering the wide knowledge of the genetic basis of genodermatoses, more in vivo genetic therapy trials should be initiated, in order to provide patients with the best tailored treatment option.

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Conflict of Interest

Authors declare no conflict of interest.

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Review *Clin Ter 2023; 174 Suppl. 2 (6):243-248 doi: 10.7417/CT.2023.2494*

Bioetics Issues of Artificial Placenta and Artificial Womb Technology

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Abstract

The worldwide infertility crisis and the increase in mortality and morbidity among infants, due to preterm births and associated complications, have stimulated research into artificial placenta (AP) and artificial womb (AW) technology as novel solutions. These technologies mimic the natural environment provided in the mother's womb, using chambers that ensure the supply of nutrients to the fetus and disposal of waste substances through an appropriate mechanism. This review aims to highlight the background of AP and AW technologies, revisit their historical development and proposed applications, and discuss challenges and bioethical and moral issues. Further research is required to investigate any negative effects of these new technologies, and ethical concerns pertaining to the structure and operation of this newly developed technology must be addressed and resolved prior to its introduction to the public sphere. ***Clin Ter 2023; 174 Suppl. 2 (6):243-248 doi: 10.7417/CT.2023.2494***

Key word: *Ectogestation, artificial placenta technology, artificial womb technology, ethical concerns, infertility*

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Introduction

Premature birth is the major reason for mortality and morbidity among infants (1,2). Throughout the world, 0.4% of infants are born prematurely, i.e., before completing the normal 28 weeks gestation period (3,4). In the USA, every 1 out of 10 infants is born prematurely. Despite advances in healthcare, these premature neonates have a 17-fold greater death rate than full-term births, with 1 in 3 infant deaths in the USA due to complications associated with premature birth (5). A premature infant's organs (eyes, brain, lungs) and systems (cardiovascular, renal, and gastrointestinal) are exceptionally predisposed to hemodynamic irregularity and ventilatory scarcity, leading to a high mortality rate and long-term morbidity (6-9). In spite of all the efforts at the research and public health level, the mortality rate in preterm infants is still 17-fold higher than in full-term newborn babies, and it continues to increase in several countries (10,11). Additional concerns are the low populace in certain regions of the world and the worldwide infertility crisis (12). The quest to address these issues and reduce infant mortality has resulted in the creation of artificial placenta (AP) and artificial womb (AW) technologies, offering a favorable environment for the fetus' ectogestation. These technologies have demonstrated exceptional potential for improving clinical outcomes in very preterm infants, ever since Westin et al. created the first artificial womb by cannulating umbilical veins in 1958 (5).

The artificial uterus technology is of potential significance to women with diseased or damaged uteri, who are thus unable to conceive or carry children on their own. Also, an artificial uterus might act as an incubator for preterm infants—notably, those born before 24 weeks of gestation, the minimum for viability in conventional incubators. The development of such an incubator could be a game-changer in lowering fetal mortality and morbidity caused by preterm birth (13). Currently, the Children's Hospital of Philadelphia's EXTRA-uterine Environment for Neonatal Development (EXTEND), and Tohoku University's and the University of Western Australia's Ex-Vivo Uterine Environment (EVE) are all active applications of the technology (14). Though little progress has been made in this domain in the past 60 years, the success of experimental models has recently improved, accelerating this technology's path toward clinical application thanks to technological developments and a greater focus on mimicking uteroplacental physiology. The main steps for clinical use include miniaturization, anticoagulation, clinical risk classification, customized critical care procedures, a regulatory road, and a plan and platform to bring technology to the bedside. The remaining benchmarks for clinical translation are now being addressed by different groups. This article aims to explore the history of the AP, list the numerous current developments, and outline the major problems and ethical concerns that need to be addressed before practical application.

Understanding Artificial Placenta and Womb Technology

A better understanding of the concept of in vitro fertilization and advancements in premature infant survival have created new potential for ectogenesis. Ectogenesis refers to the implantation and full fetus development in vitro, where the presence of a synthetic or artificial uterus supports the development of embryos until viability. Artificial wombs would work by tying into an extracorporeal source of mother blood or fluid replacement. An incubated fetus would receive nourishment and oxygen from the AW, which would also be able to remove waste products. Therefore, an AP would be required to mediate the essential communications between fetal circulation and the system that would take the place of the maternal flow (13).

Historical Development and Milestones, Mechanism and Components of Artificial Placenta and Womb Technology

First-ever attempts to assist the implantation of human embryos outside of the human body were made in Italy and in New York City in 1982 and 1983, respectively (15). In 1989, it was reported that the first human embryo had been implanted in an ex vivo, isolated, and extracorporeally perfused uterus, but this sparked debate and raised ethical questions (16). Ultimately the Italian experimental program was abandoned, due to the moral concerns that were raised and to the vehement and vocal criticism from the political establishment (16). The AW is comprised of two main parts: a chamber that supplies nutrients, and another that disposes of waste. Artificial amniotic fluid is placed in the nutrient supply chamber, to keep the developing fetus alive inside the womb. A container that continuously supplies the fetus with pure, oxygenated blood until delivery is also included in the apparatus. The fetus can grow and develop at temperatures conducive to life in the womb. To maintain a consistent body temperature, pumps keep warm water flowing. Studies reveal that the mechanisms of essential nutrient supply and waste disposal can be maintained by connecting the human placenta to the artificial uterus (9,17-26). However, attempts at a viable connection were not fruitful, as placentation under these circumstances is still a problem, especially considering the need to use immunosuppressive treatments that reduce transmission of immune function to the fetus (27). By transmitting antibodies such as immunoglobulin (IgG), the placenta might provide the fetus with immunological defense (24).

One issue that needs to be overcome is that the three umbilical cord vessels that maintains blood flow between the mother and the fetus undergo a physiological occlusion, but they should stay open after the fetus is prematurely removed from the mother's uterus (28-30). The physiology of the placenta could be supported, the fetus could receive nutrients, and waste could be removed with the help of a maternal blood reserve (31). One option to fix this issue is to connect the prosthetic uterus and placenta directly to the maternal circulation (31). Using artificial blood "suppliers" and "disposers" offers the benefit of letting the fetus develop in a protected environment, where it is essentially shielded from negative consequence such as maternal sickness or exposure to pollution, alcohol, or narcotics (28,32-34). However, due to insufficient gestational immunological tolerance, autonomous systems have been unable to prevent a maternal immune response directed against the baby. Dialy-

sis is an additional method for waste removal. Extracorporeal membrane oxygenation (ECMO) has been used to oxygenate the embryo or fetus (35). Artificial intelligence (AI) may be able to provide custom nutrients to the newborn as per their needs, track physical features, and report anomalies that suggest deviations from normal gestation. Ectogenesis is a specialized discipline, wherein scientists try to mimic the exact inside-uterus conditions, so that the artificial environment in which human embryos develop closely replicates the natural one. This AW where the embryo will develop, also known as "growth pod", was first invented by Emanuel M. Greenberg in 1955 (13,36,37).

Current Research and Innovation on Artificial Placenta and Womb Technology

AP and AW models that have been developed, have been successful due to a technological evolution that copies fetal and uteroplacental biology. The AW models create an environment that surrounds the embryo or fetus with warm fluids and provides nutrition and gaseous exchange (38-40). Moreover, the AW models, maintain a balanced environment of maternal and placental hormones and growth factors, that can be used to study and reproduce normal fetus growth. Abnormal conditions, such as fetal hypoxia, can also be studied and controlled in AW models (41-44). AW models are also important for their role in highlighting and being able to correct defects in a fetus' growth. For example, if there is any placental deficiency because of a shortage of nutrition or oxygen to the fetus, it can restrict its growth. To replicate this deficiency, mechanical ventilation can be restricted, then any problem in the model can be corrected, even if the fetus is suffering from severe pulmonary, diaphragmatic, or cardiac congenital malformation(s). The role of stem cells, gene therapy, and pharmacology can be explored in these models, as these therapeutic agents can easily be delivered to the fetus without any maternal or placental barriers (14).

Advantages and Potential Applications of Artificial Womb Technology

The main target of AW is to provide a human uterus-like environment, so that the fetus can survive without suffering from the stress of preterm birth. While there is no evidence yet that supports current use of an AW (45,46), the neonatal intensive care unit that is often required for preterm infants is not always sufficient and has weaknesses that could be solved by use of an AW to reduce mortality and morbidity rates among preterm infants (45,47-49). This type of technology could also reduce the risk of Cesarean sections, a major surgery that is associated with several risks, such as excessive bleeding, wound infection, and blood clots (50-53). Moreover, AW technology allow to perform surgical operations on the growing fetus, reducing the risk of any complications for the pregnant mother, for whom these procedures are risky (21,51,54-59). Another benefit of ectogenesis is that it provides a controlled environment for the fetus to grow in, under controlled conditions of temperature, oxygen, nutrition, etc. (51,55,60). Finally, ectogenesis could be helpful for individuals and families facing difficulties with conceiving(13,47,55,60-65).

Assessing the Limits and Ethical Considerations of AW

Currently, there are several ethical and bioethical issues concerning AW, which must be addressed (68). The models used do not replicate the natural capacity of the placenta to transform pulsatile blood flow from the umbilical artery into laminar blood flow in the umbilical vein (69). Moreover, there is no clear understanding of the enduring psychological and emotional effects the use of the artificial uterus will have on parents and subsequent progeny, given the variation it introduces to the concept of childbirth (70). Concerns have also been raised about the viability of getting informed consent for these procedures, including the use of AW in clinical trials, due to the possibility of exposing study subjects to unanticipated physical and psychological damage. When establishing the parameters for experimental research, it is imperative to define specific criteria for determining the appropriate use of these technologies (71).

The utilization of artificial wombs poses an imminent risk to the notion of parenthood. Medical professionals agree that the natural mother-child connection is beneficial to both the mother and the child: a fundamental and physical trust is built between the two persons during the nine-month gestation period, a bond that is not only emotional, but also physiological, thanks to the flow of hormones. By design, the use of artificial wombs breaks this relationship, and the long-term effects of this extreme practice are difficult to predict. The use of artificial wombs thus threatens the very idea of motherhood (72). Moreover, the use of AW has the potential to reveal or intensify disparities in healthcare accessibility: the cost may pose a hindrance to accessibility, and several authors have highlighted this impediment as a possible contributor to the escalation of social inequality (60,73,74).

Prospects for Artificial Placenta and Artificial Womb Technology

Extensive research is required for the practical implementation of AP and AW technology as a healthcare procedure for humans. In the contemporary era of technological progress, it is crucial for committees to determine the medical usefulness of a particular technology and differentiate it from innovative experimental constituents designed for research objectives. Moreover, it is imperative to consider the potential benefits and therapeutic properties of the new experimental devices and their intended recipients. AP and AW could become common clinical procedures, similar to in vitro fertilization for infertility or fetal life support systems, but extensive bioethics research is needed to define the full array of ethical issues. AW technology is anticipated to serve as a critical infrastructure for diverse forms of fetal research. The scope of this research may include the examination of fetal development, exploration of various fetal conditions and diseases, genetic engineering, and other scientific investigations. In

order to ensure ethical conduct and safeguard the rights and well-being of subjects involved in AW-based research, it will be necessary to implement additional guidelines that govern human subject research. It is imperative to engage in ongoing evaluation and modification of established protocols, in order to remain current with the latest developments in AW technology and changing research environments. The optimization of benefits, adherence to ethical principles, and safeguarding of research participants can be achieved through the implementation of comprehensive guidelines.

Conclusions

Researchers are already working on artificial wombs that could significantly alter our understanding of pregnancy and its role in society. In light of recent advancements in this field, the ethical implications of employing artificial wombs for neonatal life support have received increased attention. The potential for increased gestational viability afforded by this technology underscores the potential for artificial wombs to be used as a means of further limiting women's reproductive autonomy. Nevertheless, ethical concerns pertaining to the structure and operation of this newly developed technology must be resolved prior to its introduction to the public sphere. Further research is required to investigate any negative effects of these new technologies. When discussing ectogenesis, it is crucial to be cautious with respect to potential constraints of ongoing scientific advancements, and to approach the subject matter with a meticulous examination of scientific literature. It is imperative to differentiate between technologies that can be categorized as currently available and those that are still in the realm of theoretical possibility.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Review *Clin Ter 2023; 174 Suppl. 2 (6):249-255 doi: 10.7417/CT.2023.2495*

Characterization of somatic mutations in the pathogenesis of lipedema

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Abstract

Background. Lipedema, a complex and enigmatic adipose tissue disorder, remains poorly understood despite its significant impact on the patients' quality of life. Genetic investigations have uncovered potential contributors to its pathogenesis, including somatic mutations, which are nonheritable genetic alterations that can play a pivotal role in the development of this disease.

AIM. This review aims to elucidate the role of somatic mutations in the etiology of lipedema by examining their implications in adipose tissue biology, inflammation, and metabolic dysfunction.

Results. Studies focusing on leukocyte clones, genetic alterations like TET2 and DNMT3A, and the intricate interplay between adipose tissue and other organs have shed light on the underlying mechanisms driving lipedema. From the study of the scientific literature, mutations to genes correlated to three main pathways could be involved in the somatic development of lipedema: genes related to mitochondrial activity, genes related to localized disorders of subcutaneous adipose tissue, and genes of leukocyte clones.

Conclusions. The insights gained from these diverse studies converge to highlight the complex genetic underpinnings of lipedema and offer potential avenues for therapeutic interventions targeting somatic mutations to alleviate the burden of this condition on affected individuals. ***Clin Ter 2023; 174 Suppl. 2 (6):249-255 doi: 10.7417/CT.2023.2495***

Key words: *Lipedema, Adipose Tissue, Somatic Mutations, Leukocyte Clones, Mitochondrial Activity, SAT, Localized Disorders of SAT*

Introduction

The exploration of human genetics has unveiled the pivotal role of somatic mutations in the genesis of various diseases, particularly those involving adipose tissue, as exemplified by the enigmatic disorder lipedema (1). Lipedema, primarily affecting women, manifests as an anomalous and symmetrical accumulation of subcutaneous adipose tissue (SAT) in the limbs (2). The peculiar cyto- and histomorphological features of adipocytes involved in lipedema are the subject of recent and extensive studies. In particular, more dysplastic or metaplastic/dysplastic features are emerging with increasing evidence alongside the well-known hyperplastic and hypertrophic aspects. Unlike obesity, lipedema induces distinctive symptoms—such as pain and vascular vulnerability—and remains unresponsive to conventional weight loss methods (3,4). Despite its perceived rarity, studies propose an incidence of 8% to 17% among adult women, indicating a quite prevalent presence (4). The onset of lipedema often coincides with puberty and can intensify during hormonal transitions like pregnancy and menopause, hinting at potential estrogenic involvement (3,5). While genetic predisposition is suggested, with a reported positive family history of lipedema in 64% of affected women, the inheritance pattern remains ambiguous, oscillating between X-chromosome-linked dominance and autosomal dominance with sex restriction (5).

Initiating the landscape of molecular exploration, the first gene implicated in lipedema pathogenesis was Aldo-Keto Reductase 1C1 (AKR1C1), demonstrated to be mutated in a familial context (5). This spotlight on progesterone metabolism hints at hormone-mediated pathways that might underlie the development of this condition (6). The aim of

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this review is to increase our understanding of the intricate molecular origins of lipedema, facilitating the formulation of novel preventive, diagnostic, and therapeutic strategies, which is crucial for managing such a complex disease. This endeavor employs a specific approach, aiming to unveil potential somatic mutations within the aberrant adipose tissue of lipedema patients, thereby unearthing latent non-germinal genetic triggers for the disorder's emergence (7).

Exploring Hormonal Influence: AKR1C1 Mutation and Hormones

Investigations into the genetic aspects of lipedema have uncovered mutations within affected families, notably highlighting the involvement of AKR1C1 in progesterone metabolism. Notably, this marks the first gene to be correlated with lipedema (8). This observation suggests the potential influence of hormonal factors in the development of lipedema, which aligns with its worsening during hormonal fluctuations like pregnancy and menopause (9). This interplay between genetic susceptibility and hormonal changes adds intricacy to our understanding of the origins of lipedema (2). For instance, a study by Steckelbroeck et al. revealed AKR1Cs' ability to catalyze the reduction of DHT into both 3alpha- and 3beta-Diol, shedding light on its role in steroid metabolism (10). Lewis et al. explored enzyme activity differences in tumorous and non-tumorous breast tissues in relation to progesterone metabolizing enzyme gene expression (6). They used semi-quantitative RT-PCR to assess mRNA expression ratios of various enzymes, including AKR1C1, in breast tissues.

Furthermore, Michelini et al. reported on a family with sex-limited autosomal dominant nonsyndromic lipedema, reinforcing the genetic basis of the condition and its potential inheritance patterns (8). Child et al. proposed that the genetic nature of lipedema could involve X-linked dominant or autosomal dominant inheritance with sex limitation (11). This exploration of genetics forms the foundation of a continuing education activity, aimed at equipping healthcare professionals with the knowledge to better manage lipedema patients—including strategies for diagnosis, pathophysiology, and treatment (12). In parallel, research on AKR enzymes sheds light on their roles in adipocyte functions and metabolic pathways. Notably, human aflatoxin B(1) aldehyde reductase (AKR7A2), aldehyde reductase (AKR1A1), aldose reductase (AKR1B1), dihydrodiol dehydrogenase 1 (AKR1C1), and chlordecone reductase (AKR1C4) have been extensively studied, revealing distinct catalytic abilities and tissue distributions (13). These enzymes, as part of the aldo-keto reductase (AKR) superfamily, play key roles in fat deposition and hormone metabolism, with AKR1C's heightened activity linked to subcutaneous fat accumulation and altered steroid metabolism.

Moreover, genetic studies have identified a missense variant in AKR1C1 through whole exome sequencing in lipedema patients, potentially influencing reduced progesterone metabolism and contributing to subcutaneous fat depositionc(14). This implicates AKR1C1 as a candidate gene for nonsyndromic lipedema. Recognizing the limitations of standard weight loss methods for lipedema patients, research explored the benefits of a modified Mediterranean diet (mMeD), revealing substantial weight loss and improved quality of life for both lipedema patients and controls, suggesting its potential as an effective treatment approach (15).

Overall, the intricate relationship between genetics, hormones, and metabolism continues to uncover new insights into lipedema, influencing both diagnosis and treatment strategies.

Mitochondrial Dynamics in Lipedema, Obesity, and Metabolic Dysfunction

Somatic mutations, which accumulate over an individual's lifetime, have recently taken center stage in understanding the pathogenesis of lipedema, with implications extending to mitochondrial function and activity (16). Mitochondria, recognized as pivotal regulators of metabolic processes and adipose tissue dynamics, have been implicated in the interplay between somatic mutations, obesity, and metabolic activity (2). A significant study underscored how mitochondrial DNA methylation patterns exhibit tenuous connections with obesity severity, potentially influencing mitochondrial copy number and key gene expressions (like NRF1)(17).

Delving further, investigations into mitochondrial DNA copy number (mtDNAcn) variations and methylation within mitochondrial DNA, particularly the D-Loop, shed light on the intricate interplay between nuclear and mitochondrial genetic elements within the context of obesity (18). Notably, the study's insights elucidate a complex correlation between D-Loop and LINE-1 methylation, further strengthening the case for an integrated role of nuclear-mitochondrial dynamics in obesity (18). Shifting the focus to metabolic-associated fatty liver disease (MAFLD), the interplay between mitochondrial DNA methylation and hepatic lipid accumulation becomes evident. Findings suggest that altered mtDNA methylation could potentially drive mitochondrial dysfunction and aberrant lipid metabolism in the context of MAFLD (19). Transitioning to cardiovascular health, a pioneering investigation is emerging, studying platelet mtDNA methylation as a predictive marker for future cardiovascular disease (CVD) risk among individuals with obesity. Highlighting specific mtDNA loci (MT-CO1 nt6807, MT-CO3 nt9444, and MT-TL1 nt3254), this study presents a novel approach to enhance CVD risk prediction independently of conventional risk factors (20). As these studies collectively unravel the intricate relationships between somatic mutations, mitochondrial dynamics, obesity, and associated metabolic dysregulations, a compelling hypothesis emerges: somatic mutations might contribute to lipedema development through their impact on abnormal adipose tissue proliferation, urging further exploration of the multifaceted ties between mitochondrial function and lipedema manifestations.

Mutated Somatic Clones and Adipose Tissue Growth: A Closer Look

Considering the complex genetic basis of lipedema, somatic mutations can be one of the cause of excessive and

anoumalous adipose tissue growth, such as in PIK3CA-related hyperplasia syndrome (PROS) (21). These somatic mosaic mutations, characterized by their diverse genetic alterations, intricately disrupt endocrine balance and metabolic regulation, significantly impacting adipose tissue dynamics (22). Notably, studies have delineated how PIK3CA mutations disrupt GLUT4 membrane dynamics, subsequently perturbing insulin secretion and metabolic equilibrium (23). The convergence of these somatic mutations with lipedema's unique pathology offers a novel perspective on potential genetic triggers driving the condition's manifestation, thus unveiling multifaceted molecular mechanisms that underlie its enigmatic nature.

Ladraa et al. studied PIK3CA-related overgrowth syndrome (PROS), which results from somatic mosaic gain-of-function mutations in PIK3CA, leading to various clinical manifestations and disruptions in endocrine function. Notably, their investigation on mouse model closely recapitulated the patient phenotype, revealing underlying mechanisms such as GLUT4 membrane accumulation, alterations in the insulin feedback loop, liver IGFBP1 synthesis, and metabolic reprogramming. Importantly, the use of Alpelisib effectively counteracts adipose tissue overgrowth driven by PIK3CA mutations, and restores metabolomic abnormalities in both animal models and affected patients (24). Exploring somatic mutations in genes like AKT, PIK3CA, and PIT-1 provides intriguing insights into their roles in regulating adipose tissue mass, metabolic pathways, and associated disorders. Investigating these mutations can uncover critical mechanisms underlying conditions such as lipodystrophy and overgrowth syndromes, offering potential therapeutic avenues for these complex disorders. The regulation of adipose depot mass is crucial for maintaining energy homeostasis, with AKT playing a pivotal role in adipogenesis. Deleting adipocyte AKT in mice resulted in severe lipodystrophy, insulin resistance, fatty liver, and hepatomegaly, underscoring the significance of AKT in preserving adipose tissue mass and insulin signaling (25). Furthermore, the PI3K-AKT signaling pathway is essential for cell growth and metabolism. Researchers have identified cancer-associated mutations in the PIK3CA gene, specifically p.His1047Leu and p.His1047Arg, in individuals with congenital progressive segmental overgrowth syndromes affecting fibrous, adipose, and bone tissues. These mutations lead to heightened PI3K signaling, highlighting a potential therapeutic target for this distinctive overgrowth syndrome (26). In a separate case, a 23-year-old male diagnosed with idiopathic isolated growth hormone deficiency was found to carry a PIT-1 mutation (P24L), associated with inherited combined pituitary deficiency. This case shows a unique combination of Pit-1 mutation and lipedema within the family, shedding light on potential interactions between growth hormone and sex steroids that influence fat distribution during puberty (27). Mice lacking the Cav1 gene exhibit adipocyte abnormalities and insulin resistance. A frameshift mutation (I134fsdelA-X137) in CAV1 was found in patients with atypical lipodystrophy and hypertriglyceridemia, causing partial lipodystrophy (with fat loss affecting specific body areas) and hyperlipoproteinemia, along with additional atypical features (28). This review highlights their differences and commonalities to guide future research (29). The interaction between MMP-14 and caveolin 1 (CAV1) in adipocytes could lead to abnormal matrix processing, contributing to the enlargement of subcutaneous adipose tissue (SAT) (30). Progressive resistance training enhances insulin sensitivity and promotes the balance of adrenergic alpha 2A (ADRA2) and beta pathways within subcutaneous adipose tissue in obese individuals (31).

Leukocyte Clones and Inflammation: Impact on Lipedema

As the intricate genetic tapestry of lipedema unfolds, a notable emergence is the prominence of leukocyte clones and their inflammatory implications. The involvement of certain genes, such as TET2 and DNMT3A, marked by somatic inactivating mutations in hematopoietic cells, takes center stage (33). These mutations, exacerbating insulin resistance and triggering a surge of pro-inflammatory cytokines within white adipose tissue (33), align with a pivotal study illustrating how inactivating mutations in TET2 escalate age- and obesity-linked insulin resistance, coupled with heightened IL-1β expression in white adipose tissue (34). Given lipedema's strong association with inflammation, scrutinizing leukocyte clone contributions to this pro-inflammatory trend presents a promising avenue to unravel its etiology (35). To discern whether inherent cellular differences underpin metabolic variances between adipocyte subtypes, exploration shifts to human precursor stromal cells from diverse fat depots. This study highlights that, while gene expression and adiponectin secretion diversify, inherent metabolic characteristics endure during differentiation. Subcutaneous adipo-

Table 1. Genes correlated to adipose tissue disorders with somatic mutations.

Gene	Name	adipose tissue disorders	References
PIK3CA	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha	PIK3CA-related overgrowth syndrome (PROS)	(24, 32)
AKT1	AKT serine/threonine kinase 1	Lipodystrophy	(25)
AKT2	AKT serine/threonine kinase 2	PIK3CA-related overgrowth syndrome (PROS)	(24)
MMP-14	matrix metallopeptidase 14	Lipedema	(30)
CAV1	caveolin 1	Atypical lipodystrophy and hypertriglyceridemia	(28)
AKR1C1	aldo-keto reductase family 1 member C1	Lipedema	(8)
AKR1C2	aldo-keto reductase family 1 member C2	Nonsyndromic lipedema with AKR1C1 gene involvement	(15)

cytes showcase elevated C/EBPalpha, AP2, and adiponectin expression, whereas visceral counterparts exhibit amplified insulin-stimulated glucose uptake and signaling, affirming the retention of distinct metabolic traits through differentiation (36). The intricacies of adipose tissue expansion take on new dimensions in light of varying depot behaviors (37). Murine adipose tissue turnover rates and adipogenesis surge differently between visceral and subcutaneous depots. Adipocyte hypertrophy predominantly fuels adult fat mass expansion, accentuating adipose tissue's adaptive plasticity in metabolic equilibrium (37). Age-related somatic mutations in the hematopoietic system have been linked to clonal hematopoiesis, which is associated with an increased risk of cardiovascular disease and type 2 diabetes. Notably, inactivating mutations in the TET2 gene have been shown to exacerbate insulin resistance and metabolic dysfunction in mice, primarily driven by heightened IL-1β expression in white adipose tissue. However, this detrimental effect can be mitigated by inhibiting NLRP3 inflammasome-mediated IL-1β production (34). Moreover, research by Haring et al. has explored the intricate relationship between lifestyle, environmental factors, and clonal hematopoiesis of indeterminate potential (CHIP) as a cardiovascular risk factor. CHIP, often involving genes such as DNMT3A, TET2, JAK2, and ASXL1, has been associated with accelerated atherosclerosis and cardiovascular disease, and with underlying mechanisms tied to inflammation. While age remains a primary risk factor, emerging evidence suggests that factors like smoking, obesity/type 2 diabetes, and an unhealthy diet contribute to the occurrence of somatic mutations and the development of CHIP (38). Furthermore, investigations into adipocyte progenitors in heterozygous knockout mice that mimic TBRS phenotypes have unveiled the pivotal role of DNMT3A in regulating adipose tissue expansion, preadipocyte maturation, and inflammatory gene networks. This regulation has profound implications for energy storage and the development of obesity. Loss of DNMT3A leads to DNA hypomethylation, influencing the differentiation of adipocyte progenitors and underscoring its multifaceted role in weight regulation, as well as in preventing inflammatory obesity (39). Table 2 displays leukocyte clonal variations involved in lipedema.

Mitochondrial activity and related somatic mutations that can alter adipose tissue biology

In recent studies, researchers have explored various intricate mitochondrial changes associated with many somatic changes that contribute to altering adipose architecture. Kim et al. studied mitochondrial changes in subcutaneous adipose tissue from HIV-infected patients with lipoatrophy and matched controls, revealing mtDNA depletion, compensated mtDNA-dependent activity, increased mitochondrial biogenesis, and apoptosis induction. While mtDNA content doesn't correlate with other mitochondrial parameters, preserved mtDNA-dependent functions persist despite severe depletion (40). Giralt et al. examined the expression of marker genes related to mitochondrial function, adipocyte differentiation and metabolism, and adipokines in subcutaneous adipose tissue from healthy controls, untreated HIV-1-infected patients, and those treated with HAART, with or without HALS. Their results revealed disruptions in adipose tissue gene expression in untreated HIV-1-infected patients, implying the role of HIV-1 infection itself in eliciting alterations exacerbated by HAART, ultimately leading to HALS (41). Vernochet et al. generated a mouse model with mitochondrial transcription factor A (TFAM) disruption in adipose tissue, to explore its impact on metabolism. TFAM-deleted adipose tissue displayed altered mitochondrial function, leading to increased energy expenditure and protection against obesity, insulin resistance, and hepatosteatosis, suggesting adipose tissue mitochondria as a potential therapeutic target for obesity treatment (42). Exposure to calorie-dense foods decreased mitofusin 2 (Mfn2) expression, known for promoting mitochondrial fusion, in white and brown fat of adult animals. Reduced Mfn2 mRNA was observed in obese humans too. Knockdown of Mfn2 in adipocytes led to increased adiposity, food intake, and glucose metabolism impairment, reflecting its role in systemic metabolic regulation. Transcriptional changes in adipose tissue indicated enhanced lipogenesis and adipocyte proliferation, influencing systemic glucose utilization content (43). Capel et al. investigated MFN2-associated multiple symmetric lipomatosis (MSL), a condition marked by lipomatous masses and lipodystrophy. Patients with a homozygous p.Arg707Trp MFN2 pathogenic variant showed both lipomatous masses and a lipodystrophic syndrome, along with varying severity of Charcot-Marie-Tooth neuropathy. Lipomatous tissue exhibited mitochondrial alterations and abnormal expression of adipokines, while elevated serum FGF21 and increased fat metabolic activity were observed (44).

Pace et al. assessed the impact of highly active antiretroviral therapy (HAART) on adipose tissue mitochondrial DNA (mtDNA) depletion, mitochondrial proliferation, and adipocyte differentiation markers. They analyzed adipose tissue samples from 31 HIV-infected individuals, comparing treatment-naive participants with those on HAART. Stavudine-based HAART led to mtDNA depletion, altered mitochondrial protein mass, and reduced expression of PPARgamma, UCP2, and UCP3 mRNA, while zidovudine-based HAART resulted in similar mtDNA effects, along with

Table 2. Genetic Contributions of somatic mutations to Leukocyte Clones in adipose tissue disorders.

Gene	Name	Leukocyte Clonal Variation	Role	Reference
TET2	Tet methylcytosine dioxygenase 2	Somatic inactivating mutations in hematopoietic cells	Exacerbate insulin resistance, trigger pro-inflammatory cytokines in white adipose tissue	(34)
DNMT3A	DNA methyltransferase 3 alpha	Clonal hematopoiesis and adipocyte progenitors	Various physiological and pathological processes	(38, 39)

increased UCP1 mRNA expression, particularly in non-stavudine, protease inhibitor-containing HAART (45). Kim et al. investigated mitochondrial alterations in lipoatrophy by analyzing DNA, RNA, and protein levels in abdominal subcutaneous adipose tissue from HIV-infected patients with lipoatrophy and controls. Despite mtDNA depletion and increased mitochondrial biogenesis indicators, normal cytochrome c oxidase activity and protein levels suggest compensation. Although mtDNA content didn't correlate with other mitochondrial parameters, severe mtDNA depletion coexisted with preserved mtDNA-dependent mitochondrial functions, and oxidative stress and apoptosis were present without correlation with mtDNA content (45). Multiple symmetric lipomatosis (MSL) is a rare disorder characterized by large subcutaneous fat masses in specific trunk regions. In patients with tRNALys mutations in mitochondrial DNA, lipomas within MSL originate from brown adipose cells, as evidenced by the expression of UCP-1 mRNA in these lipomas, which is not present in normal subcutaneous fat or lipomas with different tRNALys mutations (46).

Obesity has been observed in Finnish patients with ataxia caused by mitochondrial DNA polymerase-γ mutations, and a deficient version of this polymerase has been linked to reduced subcutaneous white adipose tissue and somatic mtDNA mutations during aging (47). The decrease in mtDNA content coincides with compromised expression of adipocyte differentiation-associated genes (such as peroxisome proliferator-activated receptor-γ), lipid accumulation-related genes (including lipoprotein lipase and GLUT4), as well as adipokines like adiponectin (41,45,48). Deficient enzymatic activity of mitochondrial deoxyribonucleoside kinases (DGUOK or TK2) leads to mitochondrial DNA (mtDNA)-depletion syndromes in humans. A mouse model lacking Tk2 demonstrates growth retardation, mtDNA loss, and tissue-specific effects, highlighting Tk2's crucial role in mtDNA replication (49). In both models, there was a significant depletion of mtDNA levels in white and brown adipose tissue.

The orphan nuclear receptor estrogen-related receptor α (ERRα) is involved in fatty acid metabolism. ERRα-null (ERRα–/–) mice exhibit reduced body weight, peripheral fat deposits, and resistance to high-fat diet-induced obesity, with altered expression of genes related to lipid synthesis and metabolism, highlighting ERRα's role in metabolic regulation (50). DNMT3A and TET2 potentially impact atherosclerotic cardiovascular disease risk by modulating lipid and glucose metabolism pathways. These epigenetic regulators influence genes related to lipid homeostasis and glucose utilization, offering insights into strategies for managing and preventing cardiovascular diseases (51). Table 3 shows somatic variations involved in Lipedema.

Conclusions

In conclusion, this review underscores the significance of investigating somatic mutations in the context of lipedema. It is crucial to emphasize that genetic investigations of pathological mutations are essential not only for unraveling existing genetic factors but also for identifying novel genes associated with diseases (53-55). The genetic underpinnings of this condition present intriguing pathways for exploration, including mitochondrial activity, mutated somatic clones, and leukocyte clones. By investigating these mechanisms, we could move closer to unraveling the complex genetic factors contributing to the development of lipedema (56-58). Such understanding is pivotal for advancing targeted diagnostic and therapeutic approaches for this challenging condition.

Table 3. Genetic Variations, Molecular Roles, and Disease Associations of Key Genes related to Mitochondrial activity

Gene	Name	Adipose tissue disorders	Reference
TFAM	transcription factor A, mitochondrial	Lipodystrophy	(40)
NRF1	nuclear respiratory factor 1	Lipodystrophy	(40)
GABPA	GA binding protein transcription factor subunit alpha	Lipodystrophy	(40)
PPARGC1A	PPARG coactivator 1 alpha	Lipodystrophy and obesity	(40, 52)
PPARGC1B	PPARG coactivator 1 beta	Lipodystrophy and obesity	(40, 52)
PPRC1	PPARG related coactivator 1	Lipodystrophy and obesity	(40)
POLG	DNA polymerase gamma	Obesity	(52)
ERRα	estrogen related receptor alpha	Obesity	(52)
LPL	lipoprotein lipase	Obesity	(52)
GLUT4	solute carrier family 2 member 4	Obesity	(52)
ADIPOQ	adiponectin	Obesity	(52)
UCP1	uncoupling protein 1	Lipoatrophy	(45)
UCP2	uncoupling protein 2	Lipoatrophy	(45)
UCP3	uncoupling protein 3	Lipoatrophy	(45)
DLK1 (Pref-1)	preadipocyte factor-1	HALS-HIV-Associated Lipodystrophy Syndrome	(41)
MFN2	mitofusin 2	Obesity and lipomatosis	(43, 44)

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Conflicts of interest statement

Authors declare no conflict of interest.

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Targeting Mast Cells: Sodium Cromoglycate as a Possible Treatment of Lipedema

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Abstract

Background. Mast cells are immune cells that mediate hypersensitivity and allergic reactions in the body, secreting histamine and other inflammatory molecules. They have been associated with different inflammatory conditions such as obesity and other adipose tissue disorders. Lipedema is a chronic disease characterized by an abnormal accumulation of adipose tissue on the legs and arms, pain, and other symptoms. Mast cells may play a role in the pathology of lipedema.

Objective. Pilot study to determine levels of histamine and its metabolites in lipedema subcutaneous adipose tissue (SAT) biopsy samples, and to test sodium cromoglycate for the treatment of mast cells in women with lipedema.

Methods. Biopsies from lipedema and control SAT were collected and analyzed histologically for the presence of mast cells. Mass spectrometry was used to measure the levels of histamine, a key marker of mast cells, and its metabolites in SAT in women with lipedema and controls, and after a group of women with lipedema were administered oral and topical doses of sodium cromoglycate for two weeks.

Results. Histological examination of biopsies from lipedema patients confirmed the presence of mast cells. Metabolomic analysis revealed high levels of histamine and its metabolites in samples from women with lipedema compared to controls. Following a two-week treatment period, lipedema tissue samples exhibited reduced levels of histamine, suggesting a reduction of mast cell activity.

Conclusion. Sodium cromoglycate has the ability to stabilize mast cells and reduce histamine levels in lipedema patients, which could be useful in lowering the symptoms of lipedema. ***Clin Ter 2023; 174 Suppl. 2 (6):256-262 doi: 10.7417/CT.2023.2496***

Key words: *lipoedema, lipedema, subcutaneous adipose tissue, mast cells, sodium cromoglycate*

Introduction

Lipedema is a chronic, often debilitating disease that is characterized by excess accumulation of subcutaneous adipose tissue (SAT) in the arms and legs, accompanied by bruising and pain (1). It primarily affects women but has been reported in men as well. Lipedema often appears during times of hormone change, such as puberty or menopause, and can cause significant mobility issues and discomfort if left untreated (2,3). The exact prevalence of lipedema is not known, but it is thought to affect over 6% of women worldwide (2). It typically appears during or after times of hormone changes, such as puberty or menopause, and can become a chronic and debilitating disease if left untreated. The symptoms of lipedema vary but may include swelling and bruising of the legs and arms, discomfort and pain, and difficulty with mobility. Pain is a main symptom in lipedema and is usually associated with depression and an impaired quality of life (1,4,5).

Estrogen, being a key regulator of adipocyte lipid and glucose metabolism and of female-associated body fat distribution, is postulated to contribute to the pathophysiology of lipedema (6). Adipose tissue is a heterogenous tissue, primarily composed of adipocytes and containing a large number of immune cells—including mast cells, which are involved in allergic reactions and immune defense mechanisms Adipose tissue is a heterogenous tissue, primarily composed of adipocytes and containing a dense network of blood capillaries and a delicate component of lymphatic vessels belonging to the so-called "minimal network of both high uptake and high conduction". In addition, it is present a large number of immune cells-including mast cells, which are involved in allergic reactions and immune

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defense mechanisms (7–9). These elements are partly conveyed by the minimal blood and lymphatic capillary networks described above or reach the tissue by diapedesis. The presence of estrogen receptors on mast cells suggests that these cells are influenced by this hormone, which might thus affect their release of histamine (8,9). Therefore, lipedema could be considered, in some respects, as a condition in which there is a disturbance in the hormonal equilibrium, leading to mast cell activity within adipose tissue.

There is currently no cure for lipedema, but the condition can be managed through a combination of diet, exercise, and specialized medical interventions. Medical treatments typically used for lipedema include manual lymphatic drainage, compression therapy, and liposuction (1,2). Additionally, the Mediterranean and ketogenic diets have proved successful in inducing weight loss in lipedema patients, reducing pain and thus improving quality of life (5).

Histamine and mast cells

Histamine is a biogenic amine with several biological effects released from mast cells, a type of white blood cell found in various body tissues (10,11). Histamine is known to contribute to cell proliferation and inflammation in various tissues. The production of histamine occurs through the conversion of the amino acid L-histidine, catalyzed by the enzyme histidine decarboxylase (HDC). As shown in Heron et al.'s study, histamine receptor mRNAs and the HDC gene exhibit widespread expression in developing liver and adipose tissues in rats (10). Moreover, mast cells stimulate adipose tissue differentiation and proliferation (11). This research supports the hypothesis that mast cells and histamine might play a role in stimulating cell growth in adipose tissue, which could potentially have relevance in the context of lipedema.

Estrogen plays key role in adipogenesis

Estrogen, a hormone produced primarily in the ovaries and in smaller amounts by the adrenal gland and adipose tissue, is a vital hormone for female sexual development and for the proper functioning of the female reproductive system. Also, SAT is rich in estrogen receptors (both ERα and ERß), and thus mediate adipogenesis in SAT (6). Increased expression of ERα in preadipocytes suggests an estrogen-dependent adipogenesis in SAT (3). Estrogen and its signaling mechanisms contribute to the development of lipedema, potentially through direct effects on adipocytes and immune cells, or indirectly by influencing the brain's control centers (3). In lipedema, dysregulation of adipose tissue accumulation via estrogen signaling likely occurs by two mechanisms:

1. Altered adipocyte estrogen receptor distribution (ERα/ERß ratio) and subsequent metabolic signaling;
2. Increased release of adipocyte-produced steroidogenic enzymes, leading to increased paracrine estrogen release (6).

There is evidence that estrogens mediates the inflammatory activity of mast cells and stimulates the release of histamine (12). Histamine impacts several important physiological processes, including immune function and blood pressure regulation (13,14).

Mast cell stabilizers

Mast cell stabilizers belong to a category of medications designed to hinder the release of substances from mast cells (15). Mast cell stabilizers find application in treating various conditions in which mast cells play a role, including allergies, asthma, and certain types of cancer. Typically, these medications are used preventively to reduce the frequency and severity of allergic reactions and asthma attacks. They can be administered alone or in conjunction with other drugs, like antihistamines or inhaled corticosteroids. Notably, mast cell stabilizers have been recognized for their potential to mitigate inflammation and enhance insulin sensitivity in individuals with obesity (16). This effect arises from their ability to inhibit the release of substances like histamine from mast cells, which can impact adipocytes and other bodily tissues (17).

Sodium cromoglycate, also known as cromolyn sodium, is a mast cell stabilizer used to prevent the release of substances from mast cells (18). It is commonly employed both to prevent and to treat allergic reactions, including allergic rhinitis (hay fever), asthma, and allergic conjunctivitis (eye allergy). Sodium cromoglycate is often used preventively to reduce the frequency and severity of allergic reactions and asthma attacks and may be administered alone or alongside other medications (18–20). Notably, sodium cromoglycate is no longer under patent protection, making it available as a generic medication. Generic drugs typically come at a lower cost than brand-name counterparts, while adhering to the same standards of quality, safety, and efficacy. Sodium cromoglycate is generally regarded as safe when used as directed, though some individuals may experience fewer side effects like stomach upset, nausea, vomiting, and diarrhea (20). Aside from sodium cromoglycate, several other substances are recognized as mast cell inhibitors (21–23).

Scientific rationale

Recently, the involvement of mast cells and histamine in the development and progression of lipedema has been hypothesized (24). Histological analyses of the diseased SAT revealed an abnormal presence of mast cells, which may be involved in the pathology of the condition (9). Additionally, high levels of histamine and its metabolites that stimulate inflammation have been found in adipose tissues (8,9), suggesting that mast cell degranulation and the release of histamine may play a role in the development of excess SAT including lipedema. This has led to the suggestion that molecules with mast cell-stabilizing effects, such as disodium cromoglycate, may be useful as therapeutic targets for the treatment of lipedema. We rationalize that targeting mast cells with mast cell stabilizers could be a potential treatment for lipedema. The key points supporting the mast cell hypothesis (and histamine) as a key factor in lipedema are:

- The mast cell has receptors for estrogen, leading to the hypothesis of estrogen involvement in lipedema pathogenesis (2,3).
- High estrogen concentrations can lead to the activation of mast cells and the subsequent release of substances from these cells, a process known as degranulation. Among these substances there is histamine (12), a chemical that plays a key role in immune system responses and can cause allergic reactions, inflammation, swelling, itching, and irritation.
- Histamine can increase estrogen levels in the body, although the exact mechanisms by which this occurs are not fully understood. Some studies suggest that histamine can stimulate the production and release of estrogen from the ovaries and other estrogen-producing tissues, such as the adrenal glands and adipose tissue.
- High histamine levels in adipose tissue have been found to correlate with proliferation and inflammation of said tissue (13), which means that an increase in histamine levels within adipose tissue is associated with an increase in its size or mass. The exact mechanism by which this occurs is not fully understood, but it is thought that histamine may stimulate the growth and proliferation of adipose tissue, leading to an increase in its size.

Materials and methods

Patient recruitment

Patients diagnosed with lipedema according to Wold's criteria were recruited and classified into four stages, based on the severity of their symptoms. All patients were fully informed of the risks and benefits of participating in the study, and they provided written informed consent for their participation. The study was conducted in accordance with the ethical guidelines outlined in the Declaration of Helsinki and received ethical approval from the Ethical Committee of Azienda Sanitaria dell'Alto Adige, Italy.

Biopsy and target metabolomic examination

A total of 44 lipedema SAT biopsies and 18 non-lipedema control SAT biopsies were analyzed for this study. Three lipedema participants received a treatment regimen consisting of twice daily oral doses of sodium cromoglycate (500 mg) for 15 days, as well as a topical cream containing 4% sodium cromoglycate. This treatment was administered off-label, as sodium cromoglycate is not currently approved for the treatment of lipedema. The effects of this treatment on mast cell activation were evaluated as part of the study.

Liquid Chromatography-Mass Spectrometry (LC-MS) Analysis

Metabolomic analysis was performed using liquid chromatography (LC) coupled with mass spectrometry (MS). LC separation was carried out using a Phenomenex Jupiter C18 column (50 x 2.1 mm, 5 µm particle size). Binary gradient elution with mobile phases consisting of water with 0.2% formic acid (mobile phase A) and acetonitrile (mobile phase B) was employed. The LC system was coupled to an HCT Ultra high-capacity ion trap mass spectrometer equipped with an electrospray ionization (ESI) source. The mass spectrometer was operated in both positive and negative ion modes. Mass spectrometry analysis was performed to measure the levels of histamine and its metabolites 1-Methylhystamine and Imidazole Acetaldehyde. Data are presented as average +/- standard deviation.

Results

Analysis of the lipedema adipose tissue samples revealed elevated levels of histamine and its metabolites compared to the non-lipedema control SAT samples (Table 1 and 2). Treatment of 3 lipedema patients with disodium cromoglycate for two weeks, resulted in tissue biopsy samples with

Table 1. Tissue levels of mast cell compounds in the tissue of people with lipedema and controls

	Lipedema tissue (n=3)	Control tissue (n=4)	Lipedema tissue of patients treated with sodium cromoglycate (n=3)
Histamine (pg/mg)	18.00 ± 16.70	0	0
Histidine (pg/mg)	449.00 ± 389.66	29.75 ± 21.82	20.67 ± 17.00
1-Methylhystamine (pg/mg)	3.33 ± 5.77	1.50 ± 3.00	13.67 ± 10.02
Imidazole Acetaldehyde (pg/mg)	5.50 ± 7.78	1.25 ± 2.50	0

Table 2. Levels of histamine and its metabolites in the adipose tissue from 44 participants with lipedema and 18 controls.

	Patients (n=44)	Controls (n=18)	P-value
Histamine (pg/mg)	3.00 ± 0.61	1.38 ± 0.26	1.98E-21
Histidine (pg/mg)	362.32 ± 54.49	173.99 ± 33.84	8.34E-22
1-Methylhystamine (pg/mg)	1.92 ± 0.36	0.95 ± 0.15	3.47E-22
Imidazole Acetaldehyde (pg/mg)	5.11 ± 0.81	2.47 ± 0.45	2.15E-22

reduced levels of histamine similar to levels found for the control tissue (Table 1). This suggests that the mast cell stabilizing properties of disodium cromoglycate may be effective in reducing tissue levels of histamine, potentially improving the symptoms of lipedema. Control tissue samples had normal levels of histamine (Table 1 and Table 2), indicating that the higher histamine levels may be an abnormal finding in the SAT of lipedema participants. These findings support the hypothesis that mast cells and histamine may be involved in the pathology of lipedema, suggesting that targeting these pathways may be a promising approach for the treatment of the disease.

Discussion

Lipedema is a distressing fat disorder that primarily affects women, typically emerging during hormonal shifts like puberty, pregnancy, and menopause (2,3,6). Despite a growing awareness of the condition, the underlying causes and factors that trigger or worsen the pathophysiology of lipedema remain unclear. There is evidence that people with lipedema are more likely to a have positive family history, suggesting a heritable component (25,26). Recently, researchers discovered the first gene associated with lipedema: AKR1C1, a gene encoding the enzyme aldo-ketoreductase (27), which also plays a role in progesterone metabolism.

There is substantial evidence suggesting that estrogen could be a key factor in the onset of lipedema, given its predominant impact on women during hormonal changes (3,6). It's important to note that typical adipose tissue contains a large population of mast cells that express estrogen receptors (7,9). Estrogen has the potential to activate mast cells increasing calcium signaling, and thus stimulating mast cells degranulation and release of histamine and other cytokines (8,9,14). Mast cells degranulation releases also growth factors, which in turn stimulate the growth and proliferation of adipocytes, as well as further increasing estrogen production (9,14). Thus, the increased estrogen production and mast cell activation in adipose tissue can be self-perpetuating, leading to a vicious cycle of inflammation and tissue proliferation. Therefore, lipedema can be seen as a condition characterized by an imbalance in hormonal regulation and altered mast cell activity within adipose tissue. Although there is no permanent cure for lipedema, mast cells have been extensively targeted to treat pain and complications connected to disease (28). Pharmacological stabilization of mast cells has gained popularity over the years, as it improves insulin sensitivity, adiposity, and obesity, also preventing weight gain (15,16). Cromolyn Sodium has been widely reported for its mast cell stabilizing properties in different allergic conditions to mitigate histamine levels (18,23).

In this study, we investigated levels of histamine and the impact of sodium cromoglycate treatment in reducing histamine and its metabolites in lipedema patients. To this purpose, we analyzed a total of 44 adipose tissue biopsies from lipedema patients and 18 healthy control tissues. A preliminary histological evaluation confirmed the presence of mast cell infiltration, suggesting a possible role in disease development (Supplementary figures 1-4). Additionally, the metabolomic analysis showed increased levels of histamine and its metabolites in adipose tissue of lipedema patients (Tables 1 and 2). Three lipedema patients were treated with disodium cromoglycate and showed significantly lower levels of histamine, suggesting the mast cell stabilizing properties of this compound. In this scenario, we could view lipedema as a condition where there is a disruption in the hormonal balance within the adipose tissue, resulting in an overproduction of estrogen. This excess estrogen triggers the activation and degranulation of mast cells, which becomes a pivotal factor in the onset and progression of the condition. The resulting inflammation and tissue growth from mast cell activation can lead to the typical symptoms of lipedema, including discomfort, increased adipocyte volume, and limb enlargement (2). Many of the manifestations associated with lipedema can be linked to the activation of mast cells within adipose tissue (29). The expansion of adipose tissue leading to limb enlargement may be attributed to the inflammation and tissue damage arising from mast cell activation. Furthermore, the discomfort, inflammation, and other sensations commonly reported by individuals with lipedema may also be connected to the presence of activated mast cells within the affected tissues (14,28).

Although the study has few limitations, such as the small cohort of patients and the need for additional measurements, mainly in the histological analyses, this study provides evidence that mast cells and histamine may be involved in the pathophysiology of lipedema, a chronic and devastating disease that currently has no cure. The preliminary histological findings and the metabolomic analysis of SAT biopsies of lipedema patients, and the effects of disodium cromoglycate support the hypothesis that targeting these pathways may be a promising approach for treating lipedema.

Conclusion

The findings of this study provide insight into mast cells and histamine secretion in adipose tissue in lipedema development. We have analyzed the presence of mast cells and histamine levels in tissues of lipedema patients. Based on these findings, we believe that molecules with mast cell stabilizing effects, such as sodium cromoglycate, hold potential for mitigating the pain and complications associated with lipedema. Further research is imperative to enhance our comprehension and to develop more efficient treatments.

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Conflicts of interest statement

Authors declare no conflict of interest.

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Supplementary Material

Targeting Mast Cells: Sodium Cromoglycate as a New Possible Molecule to Treat Lipedema

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Supplementary materials and methods

Histological examination

Deep tissue sampling was performed by fibro-lipo suction with canula to obtain adipose tissue specimens. The adipose tissue was formalin-fixed and paraffin-embedded; 25 serial micro section were obtained and some of them (1-5-10 etc) stained with Hematoxylin-Eosin (H&E) allowed them to be correctly highlighted at high magnification and on very thin sections the Mast Cells due to the granule-rich cytoplasm (1). Mast cells can be observed in the interstitium between adipocytes or close to the wall of capillary vessels or lymphatic vessels. On conventional histological preparation, mast cells were identified by methods related to metachromasia. Mast cells gave a positive or negative Periodic Acid-Schiff (PAS) reaction depending on whether they contain heparin in the monosulfuric or disulfuric form.

Specific and sensitive staining of mast cells was achieved by immunohistochemistry with the CD117 antibody (Roche Diagnostics Corporation, Indianapolis, IN, USA) that highlights the proto-oncogene KIT, a growth factor for mast cells (2). Finally, mast cells were analyzed with monoclonal antibodies against tryptase (Bio SB, Santa Barbara, CA, USA). Tryptase is a serine protease produced uniquely in the granules of the mast cell cytoplasm, used as an indicator of their activation and inflammation. It is released during allergic episodes by mast cells, along with other substances such as leukotrienes, prostaglandins, and histamine (3).

Supplementary results

Tissue samples from the lipedema patients identified the presence of mast cells, indicating a potential role for these cells in the development and progression of the disease (Figure 1-4).

Biopsies were further evaluated histologically.

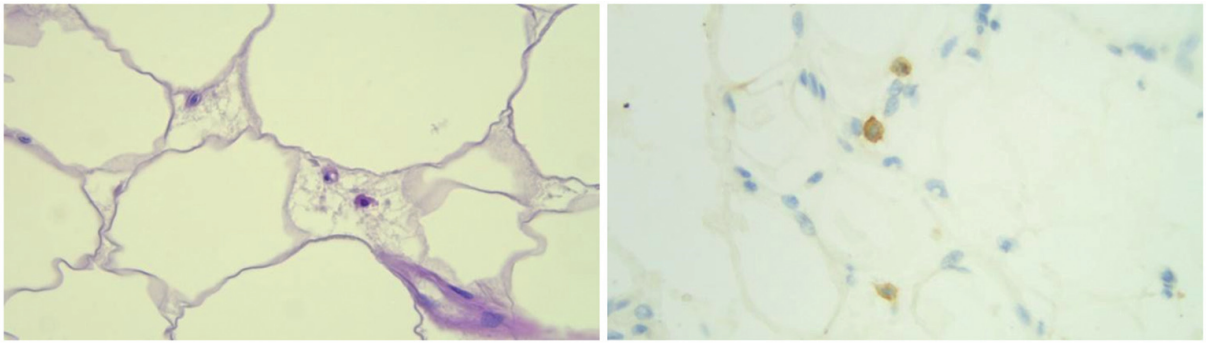

Figure 1 and 2. 1. Staining with Hematoxylin Eosin. 2. Immunohistochemistry with mAb CD117

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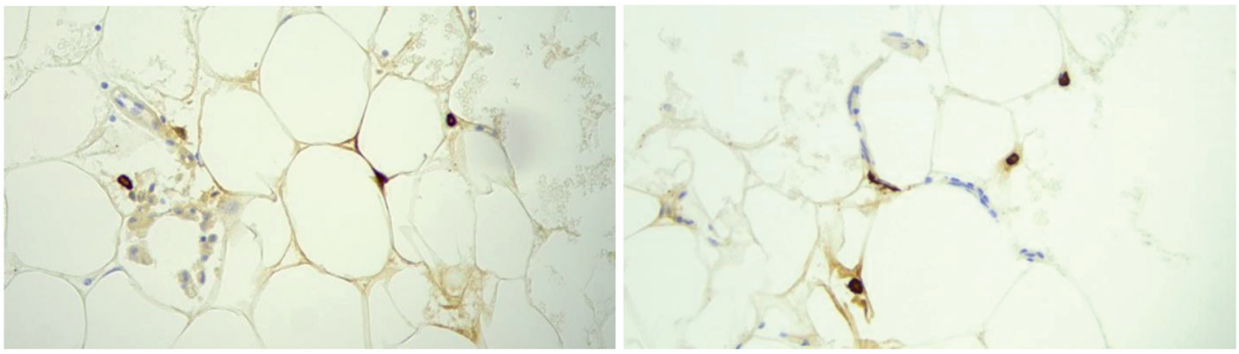

Figure 3 and 4. 3. Immunohistochemistry with mAb tryptase. 4. Immunohistochemistry with mAb tryptase

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The application of next generation matrix in the calculation of basic reproduction number for COVID-19

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Abstract

Background. Infectious diseases are disorders caused by microorganisms such as bacteria, viruses, fungi, or parasites. Many organisms live in and on our bodies. They are normally harmless or even helpful. However under certain conditions, some organisms may cause disease. Infectious diseases are also called contagious diseases due to the fact that they can be passed from person to person. Some are transmitted by insects or other animals. COVID-19 is an infectious disease that has "pervaded" the whole world during the last three years. The World Health Organization (WHO) has declared COVID-19 a Public Health Emergency of International Concern.

Methods. In this paper, we will study the outbreak of this pandemic in Albania based on some mathematical models, such as SIR, SIRD, and SEIRD. We will present a detailed analysis of these models and also demonstrate how they can be used to predict the spread of infectious diseases. More precisely, we will see the spread of COVID-19 in our country, Albania. Software such as MATLAB and RStudio will be used to do this. The data that we will use when working with these programs is taken from the Institute of Public Health, Tirana, Albania.

Results. We've developed an application utilizing actual data to estimate SEIRD model parameters. It's able to compute the basic reproduction number and, more significantly, provides forecasts on the disease's progression.

Conclusions. Our aim is to calculate the Basic Reproduction Number, using the Next Generation Matrix, and use it to see the future of the disease. This is the average number of new infections generated by an infected individual. A large value indicates that the infection is transmitted very quickly. We will try to calculate what the values of Basic Number Reproduction have been over different time periods.***Clin Ter 2023; 174 Suppl. 2 (6):263-278 doi: 10.7417/CT.2023.2497***

Key words: *COVID-19, SIR, SIRD, SEIRD, MATLAB, RStudio, Basic Reproduction Number, Next Generation Matrix*

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Introduction

COVID-19, a disease caused by the infection of SARS-CoV-2 coronavirus, can manifest as asymptomatic or be accompanied by symptoms such as fever, cough, shortness of breath, and gastrointestinal discomfort. In some cases, especially in the elderly and immunocompromised individuals, coronavirus infections can progress to severe pneumonia, ultimately leading to the patient's death (1,2). 10-20% of COVID-19 patients continue to experience COVID-like symptoms, such as fatigue, cognitive impairment, and breathlessness, even two months after recovering from the virus, developing the long-COVID syndrome (3). COVID-19 transmission methods have been categorized as direct and indirect. Direct routes of SARS-CoV-2 bioaerosol transmission include saliva droplet nuclei suspended in the indoor atmosphere, as well as maternal-to-infant transmission. The indirect mode involves transmission via fomites, which are contaminated surfaces on nearby furniture and fixtures that can lead to infection upon contact. Nasal epithelial cells have been identified as the primary entry point for SARS-CoV-2, as the virus's spike protein is cleaved by proteases. Subsequently, the spike protein of SARS-CoV-2 binds to angiotensin-converting enzyme-2 (ACE2) receptors on the cell membrane, facilitating access to host cells in the nasal and upper airway passages (1,2). Various treatments have been suggested for individuals infected with the virus, including antiviral drugs and monoclonal antibodies (1,2), while the most fast and effective methods used to combat the COVID-19 pandemic have been showed to be isolation and social distancing. The effectiveness of these approaches has been proved by statistical and computational analyses. These analyses allowed epidemiologists and virologists to assess how the virus's spread evolved over time and how the numbers of infections, recoveries, and deaths fluctuated daily (4).
The COVID-19 pandemic brought our attention back to infectious diseases; the consequences of infectious diseases can be prevented by taking measures to curb their spread.
The basic reproduction number is a coefficient that shows the number of people who can be infected by a sick person. This coefficient is a positive number, and it can be more than one, less than one, or equal to one.

1. $R_0 < 1$, means that the number of new cases will be reduced.

   When the effective reproduction number is small, it means that the rate of transmission is small; on the contrary, a large value of this coefficient indicates that the virus is spreading rapidly.

2. $R_0 = 1$, means that the number of new cases is stored within a certain range.
   Which means that the number of new cases is kept within a certain value. In the case where each infected individual infects another individual. These individuals may recover (best case) or die (worst case). Despite this, each individual infects another individual. So we have had a stable number of infections over time.

3. $R_0 > 1$, means that the number of new cases will increase and that preventive measures are not being complied with or are not sufficient.

   The value of the Basic Reproduction Number greater than one means that the number of new cases will increase and that prevention measures are not being respected or are insufficient.

The basic reproduction number is normally calculated based on three parameters, duration of contagiousness after infection, the likelihood of infection between the affected individual and the

susceptible individual and the contact rate. This coefficient has different values for different types of infectious diseases [5] (see Table 1).

Table 1. The value of the basic reproduction number in some different diseases.

Infectious diseases	Basic Reproduction Number	Infectious duration
	5	60
VDUV		v
P hdvdhv	3	v
F klf nhqsr {		v
P xp sv	3	

There are some models that can be used to find the basic reproduction number based on SIR, SIRD, and SEIRD models [10]. Regarding to the infection spreading we can use different models, for example, we can use the SIR model to see how fast mumps and chickenpox are spreading since we know that the fatality rate is very low (1.6–3.8 per 10,000 individuals) and the SIRD and SEIRD models to see how fast SARS is spreading since we know that the fatality rate is significant.

Materials and methods

The three models SIR, SIRD, and SEIRD consist of dividing the population into several categories. To find the number of individuals in each of the populations, we involve a system of differential equations, derived from the next-generation method. The method to compute the basic reproduction number using the next-generation matrix is given by Diekmann, Van den Driessche, and Watmough. To calculate the basic reproduction number by using a next-generation matrix, the whole population is divided into n compartments in which there are m infected compartments. Let $x_i, i = 1,2,3 \dots m$ be the number of infected individuals in the i^{th} infected compartment at the time t. Let $F_i(x_i)$ be the rate of appearance of new infections in compartment i and let $V(x_i) = V_i^-(x_i) - V_i^+(x_i)$ where $V_i^+(x_i)$ is the rate of transfer of individuals into compartment i by all other means and $V_i^-(x_i)$ is the rate of transfer of individuals out of the i compartment. The difference $F_i(x_i) - V_i(x_i)$ gives the rate of change of x_i:

$$\frac{dx_i}{dt} = F_i(x_i) - V_i(x_i) \tag{1.1}$$

Assuming that F_i and V_i meet the conditions outlined by Diekmann et al. (1990) [6] and Van den Driessche & Watmough (2002) [7], we can form the next generation matrix FV^{-1} from matrices of partial derivatives of F_i and V_i. Specifically,

$$F = \left[\frac{\partial F_i(x_0)}{\partial x_j}\right] \tag{1.2}$$

and

$$V = \left[\frac{\partial V_i(x_0)}{\partial x_j}\right] \tag{1.3}$$

where $i, j = 1,2, \dots, m$ and x_0 is the disease-free equilibrium.
Basic Reproduction Number is the dominant eigenvalue of the matrix $K = FV^{-1}$.

The SIR model divides the population into three compartments: Susceptible, Infected, and Recovered. The parameter of this model are: infection rate, β and recovery rate, γ.
For the SIR model, the system of differential equations is:

$$\begin{cases} \frac{dS}{dt} = -\frac{\beta S(t)I(t)}{N} \\ \frac{dI}{dt} = \frac{\beta S(t)I(t)}{N} - \gamma I(t) \\ \frac{dR}{dt} = \gamma I(t) \end{cases} \quad (1.4)$$

According to the step of the Next Generation Matrix we can calculate the Basic Reproduction Number. Firstly we have to define F and V matrices. From the Next Generation Matrix we know that:

$$F = \left[\frac{\partial F_i(x_0)}{\partial x_j}\right] \text{ and } V = \left[\frac{\partial V_i(x_0)}{\partial x_j}\right]$$

where $F_i(x_i)$ be the rate of appearance of new infections in compartment i and the V_i transfers of infection from one compartment to another. So for the SIR model:

$$F_1 = \frac{\beta S(t)I(t)}{N} \Rightarrow F = \frac{\partial F_1}{\partial I} = \frac{\beta S(t)}{N}$$

$$V_1 = \gamma I(t) \Rightarrow V = \frac{\partial V_1}{\partial I} = \gamma$$

The next step of the Next Generation Matrix will be : $K = FV^{-1} = \frac{\beta S(t)}{\gamma N}$.

At the disease-free equilibrium, $t = 0, S(0) = N$ so that $K = \frac{\beta}{\gamma}$

For the SIR model the basic reproduction number will be: $\frac{\beta}{\gamma}$

The SIRD model divides the population into four compartments: Susceptible, Infected, Recovered, and Dead. The parameters of this model are: infection rate β, recovery rate γ, and death rate α.
For the SIRD model, the system of differential equations is:

$$\begin{cases} \frac{dS(t)}{dt} = \frac{-\beta S(t)I(t)}{N(t)} \\ \frac{dI(t)}{dt} = \frac{\beta S(t)I(t)}{N(t)} - \gamma I(t) - \alpha I(t) \\ \frac{dR(t)}{dt} = \gamma I(t) \\ \frac{dD(t)}{dt} = \alpha I(t) \end{cases} \quad (1.5)$$

In SIRD model the infected compartment is "I" or Infected:

$$\frac{dI(t)}{dt} = \frac{\beta S(t)I(t) - \gamma I(t) - \alpha * I(t)}{N(t)}$$

Denote $F_1 = \frac{\beta S(t)I(t)}{N}$ and $V_1 = \gamma I(t) + \alpha I(t)$

Differentiating F_1 and V_1 with respect to I we can find the matrices F and V:

$$F = \frac{\partial F_1}{\partial I} = \frac{\beta S(t)}{N} \qquad V = \frac{\partial V_1}{\partial I} = \gamma + \alpha$$

The Next Generation matrix will be: $K = FV^{-1} = \frac{\beta S(t)}{(\gamma+\alpha)N}$.

At the disease-free equilibrium, $t = 0, S(0) = N$ so that $K = \frac{\beta}{\gamma+\alpha}$

For the SIRD model the basic reproduction number will be: $\frac{\beta}{\gamma+\alpha}$

In the SEIRD model is the population divided into five compartments: Susceptible, Exposed, Infected, Recovered, and Dead. The parameters of this model are: infection rate β, recovery rate γ, incubation rate κ and mortality rate α. For this model, the system of differential equations is:

$$\begin{cases} \frac{dS(t)}{dt} = -\frac{\beta S(t)I(t)}{N} \\ \frac{dE(t)}{dt} = \frac{\beta S(t)I(t)}{N} - \kappa E(t) \\ \frac{dI(t)}{dt} = \kappa E(t) - \gamma I(t) - \alpha I(t) \\ \frac{dR}{dt} = \gamma I(t) \\ \frac{dD}{dt} = \alpha I(t) \end{cases} \tag{1.6}$$

In this model we have two infected compartments which are *Exposed* and *Infected*:

$$\frac{dE(t)}{dt} = \frac{\beta S(t)I(t)}{N} - \kappa E(t)$$
$$\frac{dI(t)}{dt} = \kappa E(t) - \gamma I(t) - \alpha I(t)$$

From these equations we define F_1, F_2, V_1 and V_2 in order to find F and V matrices.

$$F_1 = \frac{\beta S(t)I(t)}{N}\ ; \quad F_2 = 0$$

$$V_1 = \kappa E(t)\ ; \quad V_2 = -\kappa E(t) + (\alpha+\gamma)I(t)$$

Now we can find F and V matrices by partial derivatives:

$$F = \begin{pmatrix} \frac{\partial F_1}{\partial E} & \frac{\partial F_1}{\partial I} \\ \frac{\partial F_2}{\partial E} & \frac{\partial F_2}{\partial I} \end{pmatrix} = \begin{pmatrix} 0 & \frac{\beta S(t)}{N} \\ 0 & 0 \end{pmatrix} \overset{t=0}{=} \begin{pmatrix} 0 & \beta \\ 0 & 0 \end{pmatrix}$$

At the disease-free equilibrium, $t = 0, S(0) = N$.

$$V = \begin{pmatrix} \frac{\partial V_1}{\partial E} & \frac{\partial V_1}{\partial I} \\ \frac{\partial V_2}{\partial E} & \frac{\partial V_2}{\partial I} \end{pmatrix} = \begin{pmatrix} \kappa & 0 \\ -\kappa & \alpha+\gamma \end{pmatrix} \Rightarrow V^{-1} = \begin{pmatrix} \frac{1}{\kappa} & 0 \\ \frac{1}{\alpha+\gamma} & \frac{1}{\alpha+\gamma} \end{pmatrix}$$

Now the next generation matrix is:

$$K = FV^{-1} = \begin{pmatrix} \frac{\beta}{\alpha+\gamma} & \frac{\beta}{\alpha+\gamma} \\ 0 & 0 \end{pmatrix}$$

The basic reproduction number is the dominant eigenvalue of the matrix K. The dominant eigenvalue of the matrix K is $\frac{\beta}{\alpha+\gamma}$. So for the SEIRD model the Basic Reproduction Number is $R_0 = \frac{\beta}{\alpha+\gamma}$.

Results and Discussions

In the SIR model, we have to calculate two coefficients, which are infection rate β and recovery rate γ. If we have the number of individuals who have been infected and the number of individuals who have becovered over a period of time, we can calculate these two coefficients. Meanwhile, in the SIRD model, we have to calculate another coefficient, which is the mortality rate α. To calculate it, we also need the number of individuals who have died in a given period of time. For Albania, we will use the daily data from March 9, 2021, to April 20, 2022. A sample of the dataset is given in Table 2.

Table 2. Data officially confirmed with COVID-19 from the Institute of Public Health, Albania.

Date	Date_M/D/Y	Component	Albania
<OP du0		Lqihfwng	5
<OP du0		Uhfryhuhg	3
<OP du0		Ghdwk	3
0P du0	3	Lqihfwng	
0P du0	3	Uhfryhuhg	3
0P du0	3	Ghdwk	3

Using the SIR, SIRD, and SEIRD models we have calculated the basic reproduction number and predict how many infected, recovered, and dead individuals there will be in the upcoming time period. In order to calculate the basic reproduction number, we need to first calculate the model parameter [11, 12].

The parameters of the SIR model were infection rate β and recovery rate γ. To calculate these parameters, we can use the first and the third equations of the system (1.4).

$$\frac{dS}{dt} = -\frac{\beta S(t) I(t)}{N} \Longrightarrow \beta = \frac{S_i - S_{i+1}}{S_i I_i} N$$
$$\frac{dR}{dt} = \frac{\gamma I(t)}{N} \Longrightarrow \gamma = \frac{R_{i+1} - R_i}{I_i}$$

Remember that in SIR model, if N is the population, we know that $N = S + I + R$, so the susceptible population will be $S = N - I - R$.

The parameters of the SIRD model were infection rate β, recovery rate γ, and mortality rate α. To calculate these parameters, we can use the first, the third and the fourth equations of the system (1.5).

$$\frac{dS}{dt} = -\frac{\beta S(t) I(t)}{N} \Longrightarrow \beta = \frac{S_i - S_{i+1}}{S_i I_i} N$$

$$\frac{dR}{dt} = \frac{\gamma I(t)}{N} \Longrightarrow \gamma = \frac{R_{i+1} - R_i}{I_i}$$
$$\frac{dD}{dt} = \frac{\alpha I(t)}{N} \Longrightarrow \alpha = \frac{D_{i+1} - D_i}{I_i}$$

In SIRD model, if N is the population, we know that $N = S + I + R + D$, so the susceptible population will be $S = N - I - R - D$.
The parameters of the SEIRD model were infection rate β, incubation rate κ, recovery rate γ, and mortality rate α. To calculate these parameters, we can use the first, the third, the fourth and the fifth equations of the system (1.6).

$$\frac{dS}{dt} = -\frac{\beta S(t) I(t)}{N} \Longrightarrow \beta = \frac{S_i - S_{i+1}}{S_i I_i} N$$
$$\frac{dI(t)}{dt} = \kappa E(t) - \gamma I(t) - \alpha I(t) \Longrightarrow \kappa = \frac{I_{i+1} - I_i + \gamma I_i + \alpha I_i}{E_i} = \frac{I_{i+1} + (\gamma + \alpha - 1) I_i}{E_i}$$
$$\frac{dR}{dt} = \frac{\gamma I(t)}{N} \Longrightarrow \gamma = \frac{R_{i+1} - R_i}{I_i}$$
$$\frac{dD}{dt} = \frac{\alpha I(t)}{N} \Longrightarrow \alpha = \frac{D_{i+1} - D_i}{I_i}$$

In SEIRD model, if N is the population, we know that $N = S + E + I + R + D$, so the susceptible population will be $S = N - E - I - R - D$.
Based on the formulas mentioned above we will use two approaches.
In order to find the model parameters we will define:

i. $b = (\beta_1, \beta_2, \dots, \beta_t)$, b is a vector that store infection rate values.
ii. $g = (\gamma_1, \gamma_2, \dots, \gamma_t)$, g is a vector that store recovery rate values.
iii. $a = (\alpha_1, \alpha_2, \dots, \alpha_t)$, a is a vector that store mortality rate values.
iv. $k = (\kappa_1, \kappa_2, \dots, \kappa_t)$, k is a vector that stores incubation rate values.

where t is the number of days of the time-period we are taking into consideration.

In the first approach, for $\beta, \gamma, \alpha,$ and κ, we take the last component of the respective vectors b, g, a and k.
In the second approach, we calculate $\beta, \gamma, \alpha,$ and κ as the mean of the component of the respective vectors b, g, a and k.
In Table 3 we are presented the basic reproduction numbers for different time logs, the first one is 3 months, the second one is second one is 6 months, the third one is 9 months and the fourth one is 12 months. For all the time logs we have calculated the basic reproduction number using the first and second approach.

Table 3. The basic reproduction number, SIR model for Albania.

Date	R_0 (First approach)	R_0 (Second approach)
# 0# 3		7
# 0#		
# 0# 3		
# 0# 4		

Using the results of Table 3 we have performed the prediction of the Susceptible, Infected and Recovered population. In the following graphs are given the predictions using the SIR model and the fist approach (Figure 1) [9].

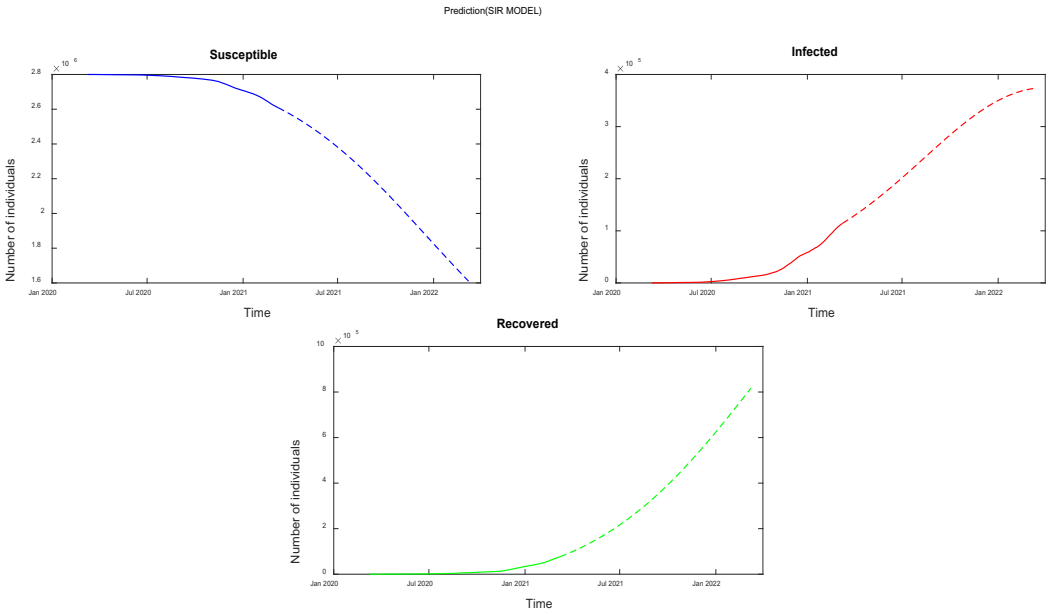

Figure 1. *The prediction of the Susceptible, Infected and Recovered using SIR model with the first approach.*

In Figure 2 are given the predictions of the SIR model using the second approach. From the graphs we see that the prediction shows some fluctuations, but at this stage we will not discuss about the accuracy.

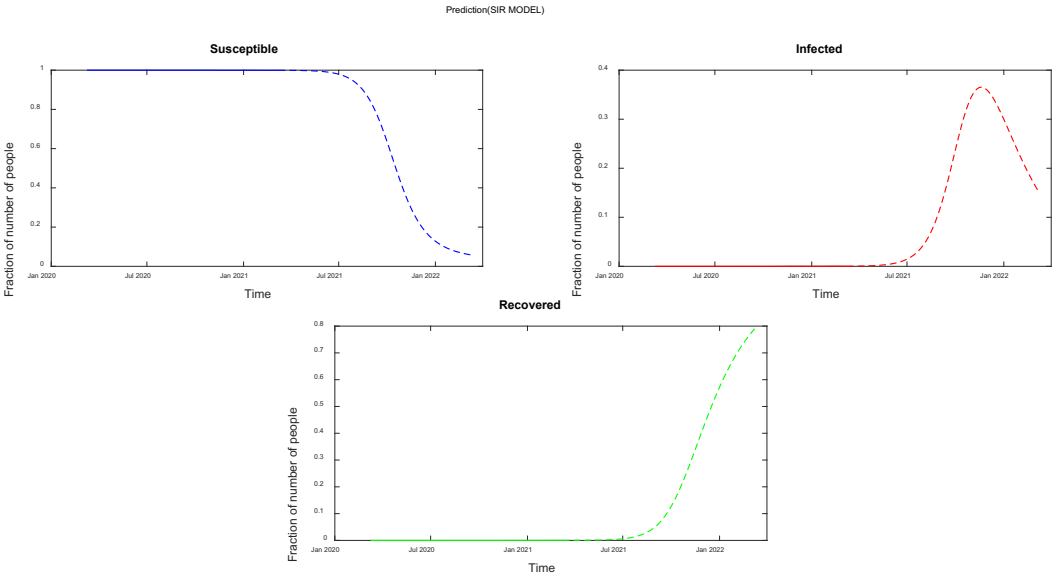

Figure 2. *The prediction of the Susceptible, Infected and Recovered using SIR model with the second approach.*

In Table 4 we have presented the basic reproduction number for SIRD model, also for both approaches.

Table 4. The basic reproduction number, SIRD model for Albania.

Date	R_0 (First approach)	R_0 (Second approach)
# 0# 3		
# 0#		
# 0# 3		
# 0# 4		

In Figure 3 and Figure 4 are given respectively the predictions using the SIRD model for each approach. Continuing in the same vain in Table 5 we have calculated the basic reproduction number for SEIRD model also for both approaches.

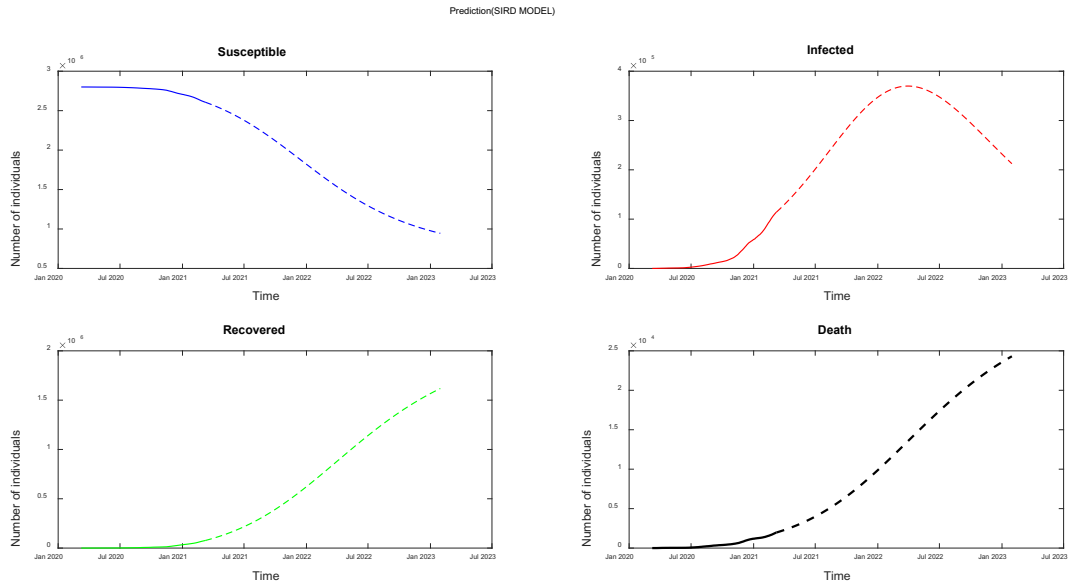

Figure 3. *The prediction of the Susceptible, Infected, Recovered and Death using SIRD model with the first approach.*

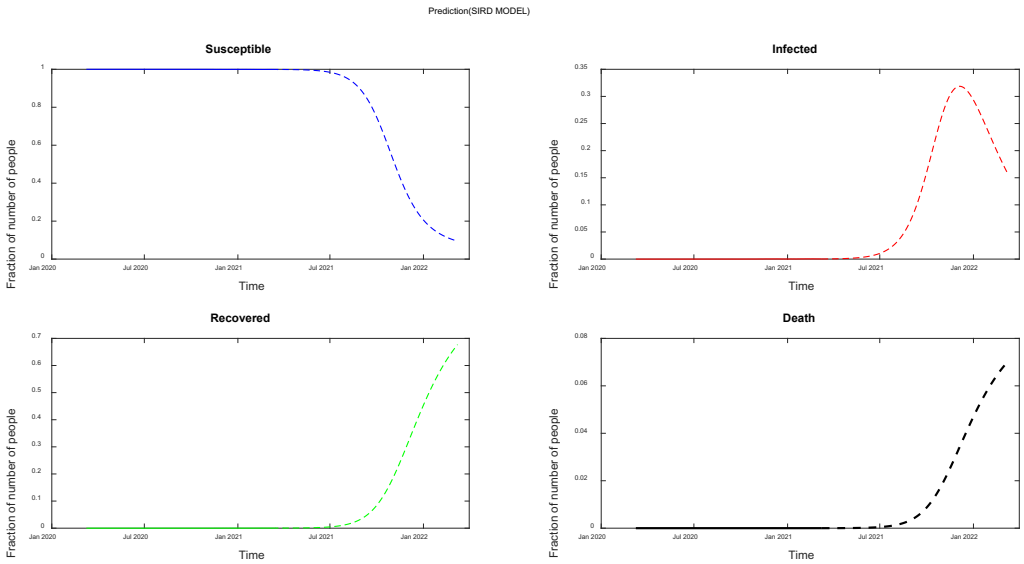

Figure 4. *The prediction of the Susceptible, Infected, Recovered and Death using SIRD model with the second approach.*

Table 5. The basic reproduction number, SEIRD model for Albania.

Date	R_0 (First approach)	R_0 (Second approach)
# 0# 3		3
# 0#		
# 0# 3		
# 0# #		

In Figure 5 we presented the prediction of the SEIRD model using the first approach. In difference from the previous model the time of the prediction is extended in order to see how the infection will evolve.

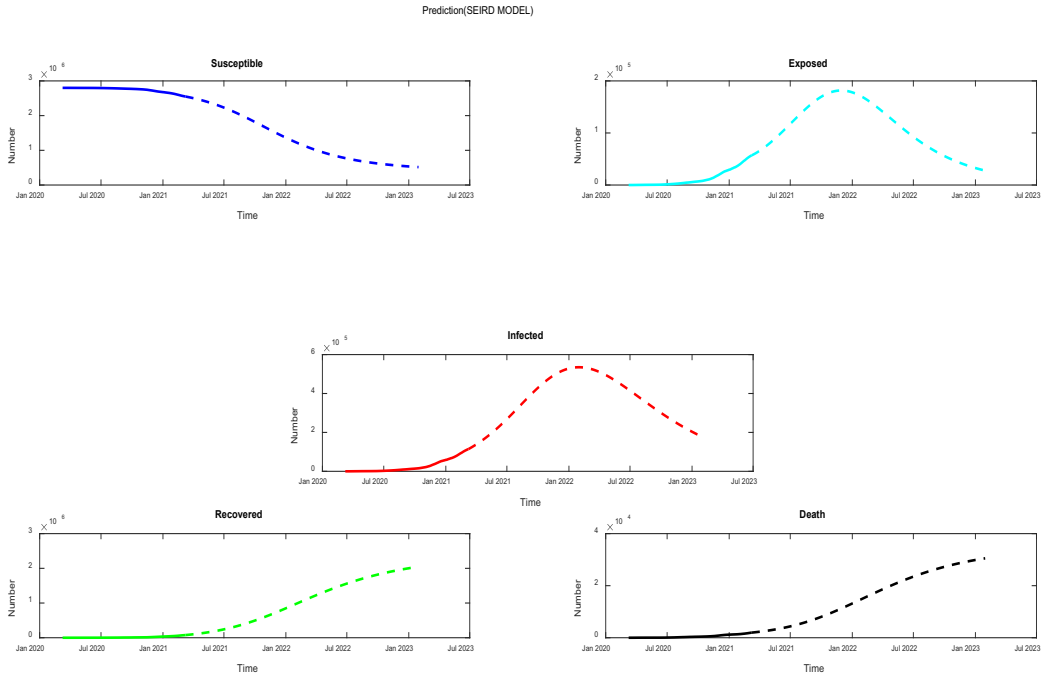

Figure 5. *The prediction of the Susceptible, Exposed, Infected, Recovered and Death using SEIRD model with the first approach.*

In Figure 6 are presented the predictions of categorical divisions of the population using the SEIRD model and the second approach. We have used the half shorter time prediction compared with the ones presented in Figure 5, but we distinguish that the fluctuations are presented in the second approach.

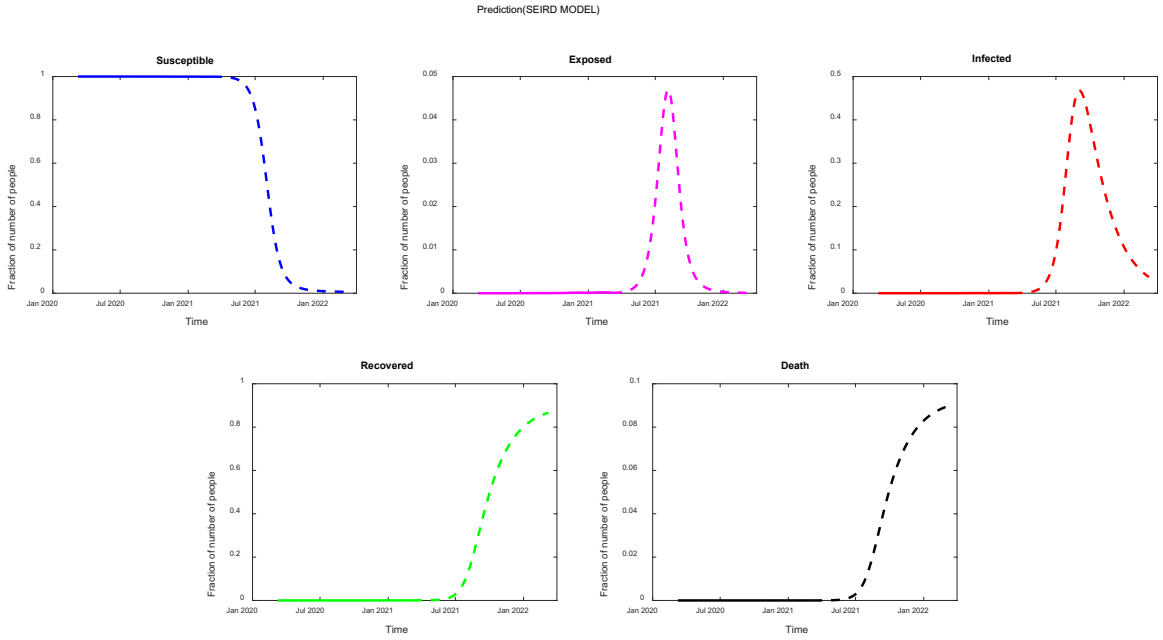

Figure 6. *The prediction of the Susceptible, Exposed, Infected, Recovered and Death using SEIRD model with the second approach.*

In the following graphs we have presented the comparison of the predictions with the real data, as the time we are studying is the past one. As it is clearly distinguished from the graphs the first approach is more accurate than the second one and from these results we come into the conclusion that the first approach is one that we shall use at the *Shiny-up* application.

From the results obtained and presented in Figure 7-9 we conclude that the predictions given from the SEIRD model using the first approach are very accurate.

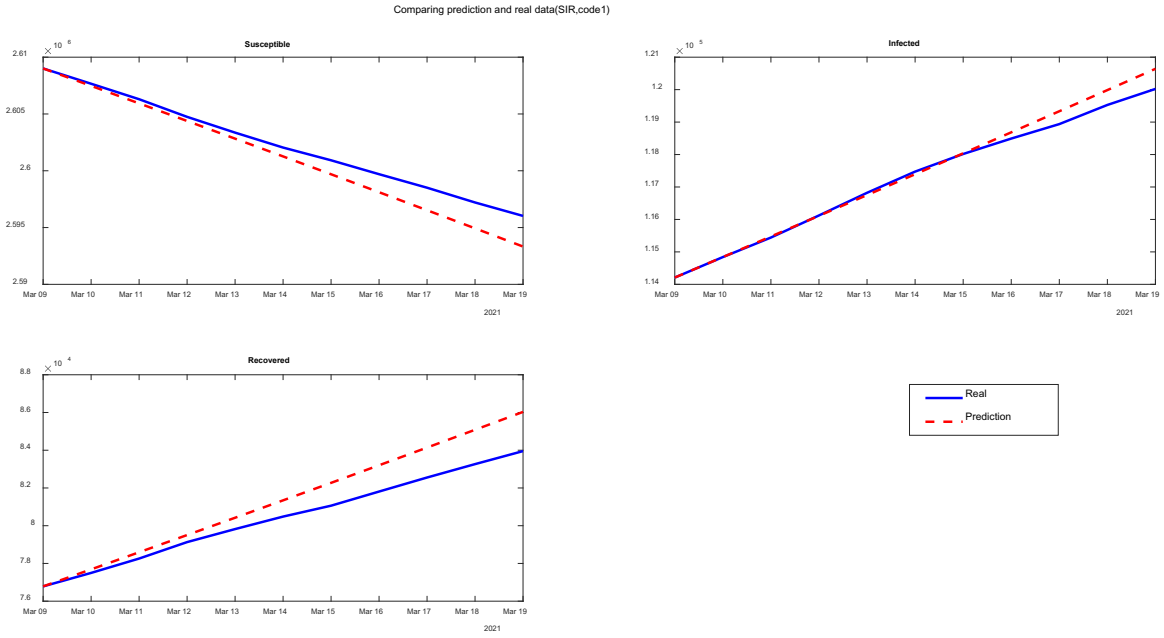

Figure 7. *The comparison of the predicted values with the real ones using SIR model with the first approach.*

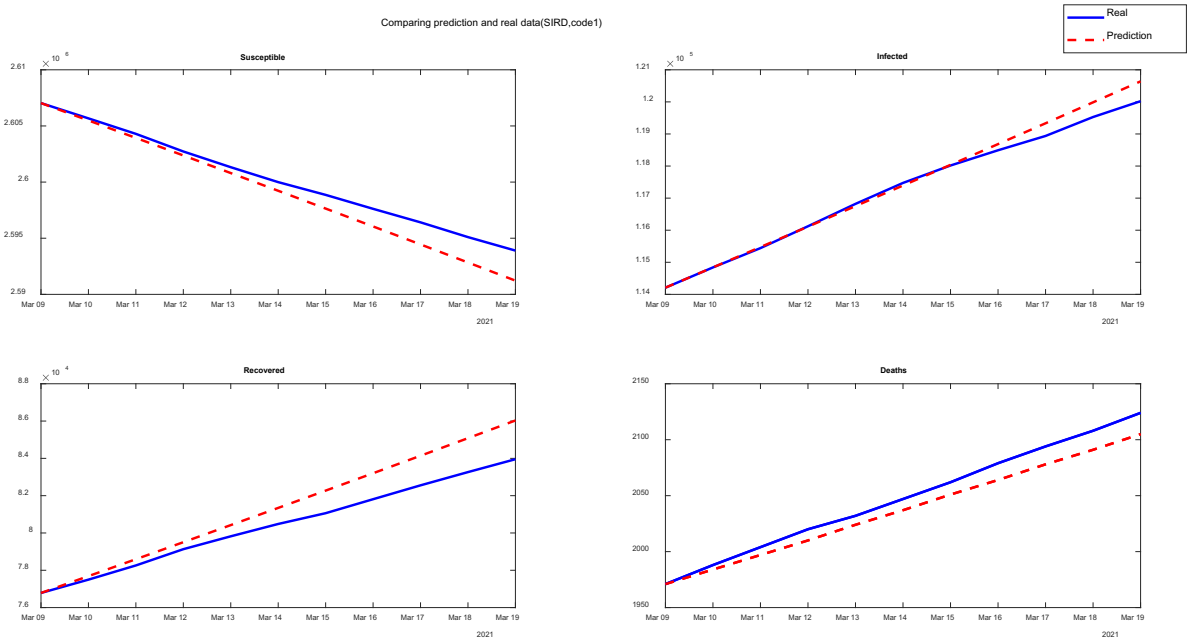

Figure 8. *The comparison of the predicted values with the real ones using SIRD model with the first approach*

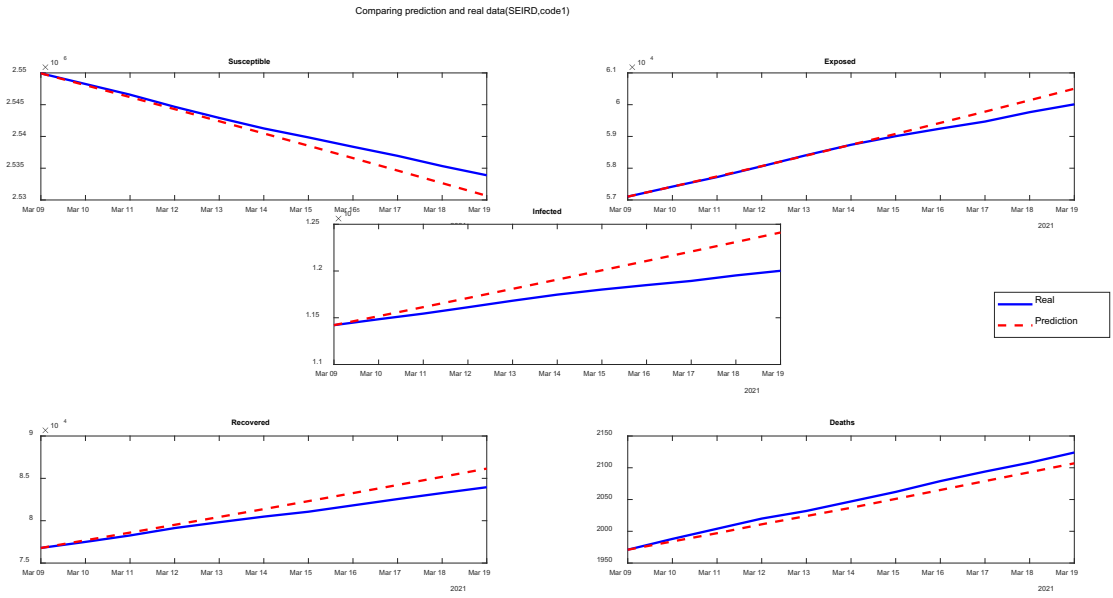

Figure 9. *The comparison of the predicted values with the real ones using SEIRD model with the first approach*

In Figure 10-12 we will compare the predictions with the real data for all the models using the second approach.

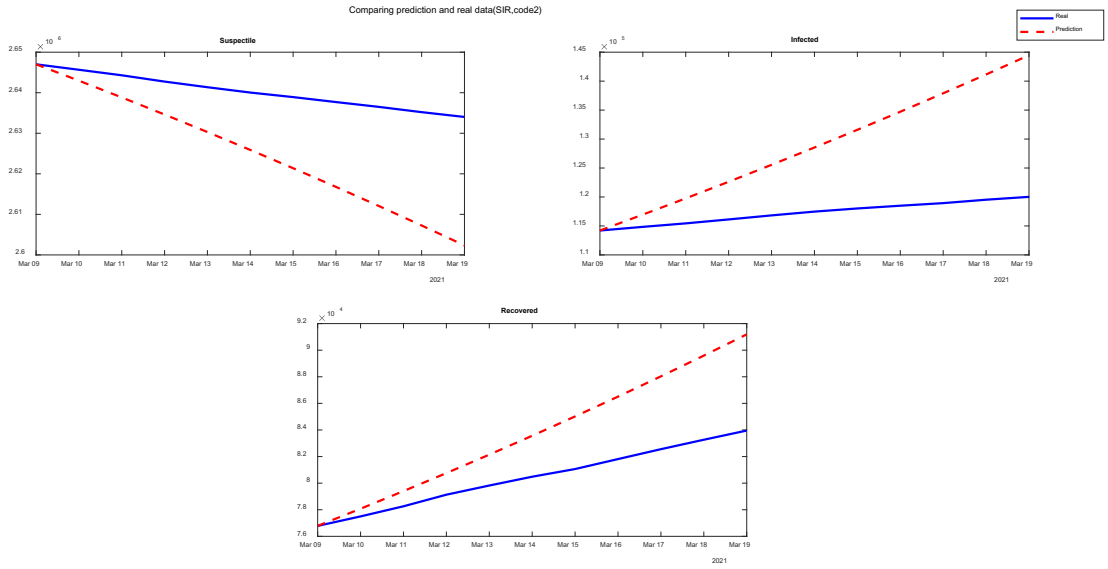

Figure 10. *The comparison of the predicted values with the real ones using SIR model with the second approach*

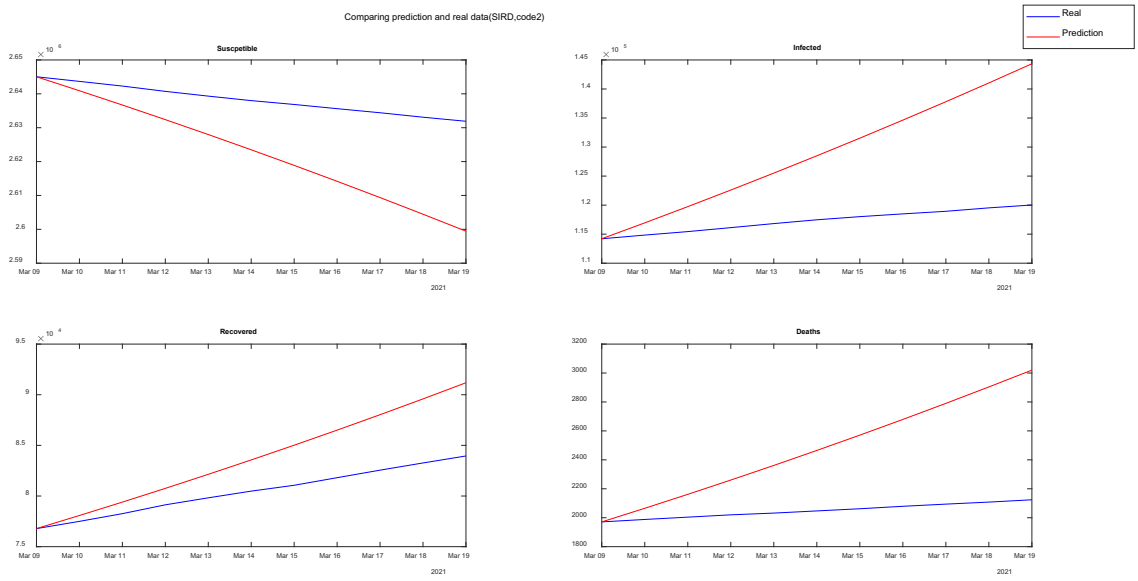

Figure 11. *The comparison of the predicted values with the real ones using SIRD model with the second approach*

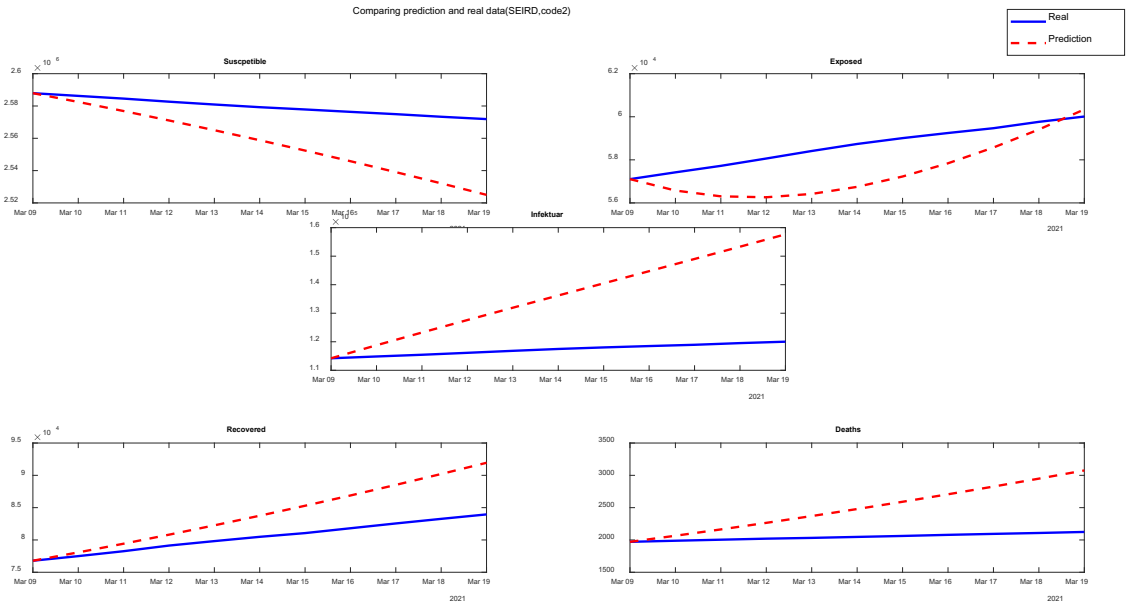

Figure 12. *The comparison of the predicted values with the real ones using SEIRD model with the second approach*

From the results obtained and presented in Figure 10, Figure 11, and Figure 12 we conclude that the second approach does not give an accurate prediction and using this fact in the application that we have built we will use the first approach and the most appropriate model for COVID-19 is the SEIRD one.

In order to give some estimations to the epidemiologists in order to take precautions and give orientations for public opinion, using this models we have built a *shiny_app* where the user (in our case, the epidemiologist) can set the values of the parameters of the SEIRD model, and the program can generate a forecast of the epidemiological situation. The same code can be modified for the SIRD model and it is applicable not only for COVID-19 but also for other infectious diseases [8].

The advantage of the application we have built is that it estimates the parameters of the SEIRD model using real data and based on these parameters it calculates the basic reproduction number and what is more importantly gives a prediction how the disease will evolve.
In the following figures we have presented just a screenshot of the application

Also, epidemiologists can estimate the values of the parameters for SIR model, SIRD model or SEIRD model if they know the number of susceptible, infected, recovered, and dead people.
In Figure 13 we have presented a screen shot of the application where the epidemiologist fill the number of susceptible, infected, recovered and dead people and the output of the apps are the parameters of the model we are interested to apply.

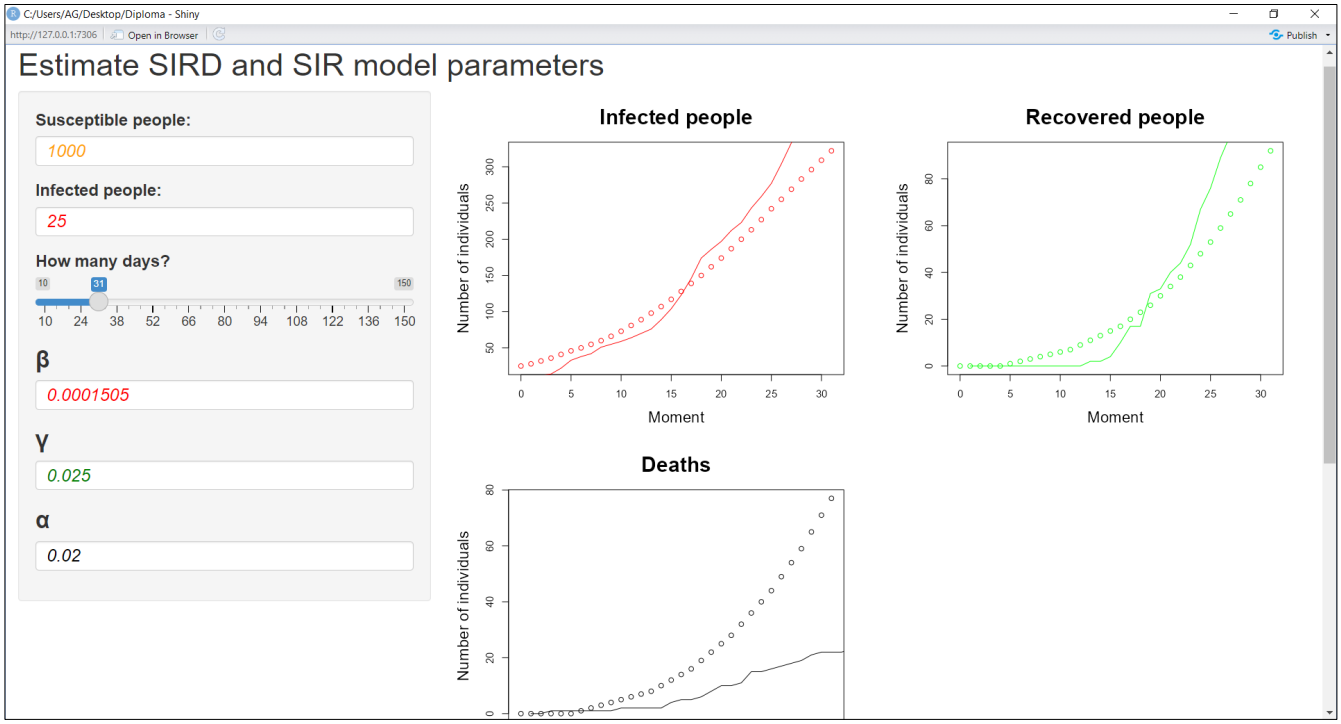

Figure 13. *The view of the application that estimates the parameters of the SIRD and SIR models.*

Once the parameters of the model are estimated they are used to construct the model with all its elements. In Figure 14 is presented the screen shot during the execution of the application.

Figure 14. *The screen shot of the Shiny_app of the SEIRD model for COVID-19.*

Conclusions

This article aimed to develop a mathematical model that clarifies and simplifies the understanding of the epidemiological growth of any infectious disease. The model, based on the structure of the SIR, SIRD, and SEIRD models, was tested using official data on the course of COVID-19 in Albania. The results obtained demonstrate that this model provides a robust foundation for epidemiological analysis and forecasting the evolution of an infectious disease with considerable accuracy. This article also offers a valuable resource for epidemiologists, in the form of an interactive application that allows to customize the parameters of the SEIRD model to forecast the epidemiological situation. Moreover, this article emphasizes that the same application can be adapted for SIRD and SIR models, demonstrating the broad applicability of this methodology, not only for COVID-19 but also for other infectious diseases. In conclusion, this article helps the understanding and forecasting of epidemiological growth in infectious diseases, offering epidemiologists and healthcare professionals important tools for planning and managing future health emergencies.

Acknowledgments
None.

Conflict of interest
The authors declare not conflict of interest.

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