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Telephone: +86-10-85381891
Fax: +86-10-85381893
E-mail: bpgoffice@wjgnet.com
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Baishideng Publishing Group Co., Limited
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FRONTIER

# Cannabinoids: Do they have the potential to treat the symptoms of multiple sclerosis?

Zubair Ahmed

Zubair Ahmed, Neurotrauma and Neurodegeneration Section, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Birmingham B15 2TT, United Kingdom
Author contributions: Ahmed Z participated in research design, conducted experiments, performed data analysis and wrote the manuscript.
Supported by The University of Birmingham
Correspondence to: Dr. Zubair Ahmed, Neurotrauma and Neurodegeneration Section, School of Clinical and Experimental Medicine, College of Medical and Dental Sciences, University of Birmingham, Institute of Biomedical Research (West), Edgbaston, Birmingham B15 2TT,
United Kingdom. z.ahmed.1@bham.ac.uk
Telephone: +44-121-4148859 Fax: +44-121-4148867
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## Abstract

This article reviews the role of cannabinoids in inhibiting neurodegeneration in models of multiple sclerosis (MS). MS is a chronic, debilitating disease of the central nervous system (CNS), induced by autoimmunity-driven inflammation that leads to demyelination and thus disconnection of the normal transmission of nerve impulses. Despite the use of an array of immune modulating drugs that restore blood brain barrier function, disability continues in patients concomitant with the loss of axons in the spinal cord. MS patients therefore suffer neuropathic pain, spasticity and tremor. Anecdotal evidence suggests that MS patients using cannabis, though illegal, achieve symptomatic relief from neuropathic pain and spasticity associated with MS. The discovery of the endogenous cannabinoid (endocannabinoid) system that naturally exists in the body and which responds to cannabinoids to exert their effects has aided research into the therapeutic utility of cannabinoids. The endocannabinoid system consists of two G-protein coupled receptors cannabinoid receptor type-1 ($CB_1$) and $CB_2$. $CB_1$ is mainly expressed in the CNS and $CB_2$ is predominantly found in leukocytes, while an increasing number of potential ligands and endocannabinoid degradation molecules are being isolated. Several studies have highlighted the involvement of this system in regulating neurotransmission and its ability to prevent excessive neurotransmitter release, consistent with a capacity to provide symptomatic relief. In summary, antagonism of the $CB_1$ receptor pathway contributes to neuronal damage in chronic relapsing experimental allergic encephalomyelitis (EAE) and suppresses tremor and spasticity. The addition of exogenous $CB_1$ agonists derived from cannabis also afforded significant neuroprotection from the consequences of inflammatory CNS disease in EAE and experimental allergic uveitis models. Although clear neuroprotective benefits of cannabinoids have been demonstrated, the unwanted psychotropic effects need to be addressed. However, manipulating the endogenous cannabinoid system may be one way of eliciting beneficial effects without some or all of the unwanted side effects.

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**Key words:** Multiple sclerosis; Axonal damage; Neurodegeneration; Neuroprotection

**Core tip:** Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system and causes disability, neuropathic pain, spasticity and tremor in affected patients. Although illegal, users of cannabis report relief from pain and spasticity, probably due to the endogenous cannabinoid system that exists. Cannabinoid receptor type-1 ($CB_1$)-deficient mice accrue greater levels of neurodegeneration and poorly tolerate inflammatory and excitotoxic insults after immune attack in a model of MS, experimental allergic encephalomyelitis. Treatment of animals affected by experimental allergic uveitis (EAU) with $CB_1$ agonists also provided significant neuroprotection from the con-

sequences of EAU, suggesting that cannabinoids may slow down neurodegeneration in MS.

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## BIOGRAPHY

Dr Zubair Ahmed received his PhD from University College London, London, United Kingdom. He completed his first postdoctoral training period at the Institute of Neurology in London before moving to the University of Birmingham to continue his second postdoctoral training period. In 2007, he was awarded an Research Councils United Kingdom Academic Fellowship, the remit of which was to develop an independently funded research group before being promoted to Lecturer in 2011. He is currently a Senior Lecturer in Neuroscience. His research interests covers numerous aspects of the molecular biology of central nervous system (CNS) axon regeneration and degeneration and his most notable contribution is to the understanding of the role of caspase-2 in apoptosis of retinal and dorsal root ganglion neurons, the contribution of an endogenous mechanism for receptor shedding, the identification of growth factors capable of promoting retinal ganglion cell survival and axon regeneration, identification of alternative neuronal receptors capable of interacting with key molecules in blocking CNS axon regeneration and the observations that cannabinoids inhibit neurodegeneration in models of multiple sclerosis (MS). His curriculum vitae lists over 50 peer-reviewed publications, 2 book chapters and numerous presentations at national and international meetings.

## INTRODUCTION

MS is an inflammatory demyelinating disease of the CNS and results in disruption to the normal transmission of nerve impulses due to lesions in the CNS[1,2]. Despite immune modulating drugs that reduce blood brain barrier dysfunction, disability often continues in patients and suggests that neurodegenerative changes are key to the progression of disease[3-5]. MS patients thus display spasticity, neuropathic pain associated with neuroinflammation, excitotoxicity and chronic neurodegeneration. It is also established that axonal/neuronal loss, which occurs early in the disease process, is an important contributor of permanent disability and is often associated with active inflammation. Disability only becomes evident when normal compensatory mechanisms are exhausted, while demyelinated axons are left vulnerable to further damage by electrical activity[6,7]. Therefore identifying neuroprotective strategies are a key goal in the fight against MS.

Although the *Cannabis sativa* plant has been used for centuries as a medicinal preparation to relieve the symptoms of inflammatory and neuropathic disorders, its use is illegal[8]. However, anecdotal accounts from MS patients indicated that cannabis might offer symptomatic relief of pain and spasticity associated with MS[9]. Plant-derived "cannabinoids" have provided important insights into the biology of cannabis and have led to a multitude of clinical trials using cannabinoids to control pain and spasticity in MS patients[10]. The main active ingredient of *Cannabis sativa* was defined in 1964 as (-)-trans-delta-9-tetrahydrocannabinol [$\Delta^9$-THC or dronabinol (international non-proprietary name)][11]. $\Delta^9$-THC is not only responsible for the majority of the pharmacological actions of cannabis but also its psychoactive effects. Numerous other cannabinoids and phytochemicals also exist in the cannabis plant including cannabidiol (CBD) which is non-psychoactive and is not a cannabinoid receptor agonist[12].

## CLINICAL TRIALS OF CANNABIS IN MS

Although many patients self-medicated with cannabis, there were few clinical studies that demonstrated reliable clinical evidence of the benefits of cannabis use in MS. Some of the first few studies on the effect of $\Delta^9$-THC were not encouraging since small sample sizes were used and $\Delta^9$-THC had no effect on objective measures, despite patients reporting subjective improvements in the symptoms of MS[13]. For example, the first systematic, placebo-controlled trial with $\Delta^9$-THC and a *Cannabis sativa* plant extract, given to 16 MS patients with severe spasticity, showed no effect of the cannabinoids on spasticity[14]. A much larger trial involving 660 MS patients receiving $\Delta^9$-THC or natural Cannabis oil or a placebo, the mean reported effect on spasticity was not significantly different between control and treatment groups[15]. However, patient-reported spasticity was reduced, agreeing with earlier studies that showed subjective improvements in spasticity related to MS[14,15]. However, in a 12-mo follow up study of 657 patients, $\Delta^9$-THC was reported to have a significant effect on the objective measures of spasticity[16]. Several later studies have also shown variable results depending on cannabis preparation, dosing regime and patient numbers, throwing into doubt the use of clinical measures of spasticity.

At present, the large number of clinical trials in MS have not clarified whether cannabis is beneficial in MS but have thrown up questions about the rating of "spasticity", route of delivery, source and dosing regime[10]. Despite these reservations, self-medicated use of cannabis continues. In a postal questionnaire involving the responses received from 110 MS patients in the South of England, 43% confirmed their use of cannabis with 68% of these patients specifically using cannabis to relieve the symptoms of MS[17]. Patients affirmed that their main reason for choosing to self-medicate with cannabis was due to pain and spasms, while a small proportion used cannabis for sleep related problems[17]. In a further study, over 90% of a cohort of 112 MS patients based in the United Kingdom and United States declared that

self-medication with cannabis improved nocturnal pain, spasms and muscular pain[18].

A recent study showed that of the 572 MS patients enrolled in a clinical study, 272 (47.6%) responded to Sativex, an oral-mucosal spray that contains $\Delta^9$-THC and CBD, treatment within 4 wk with a response that was defined as > 20% decrease in spasticity[19]. A second phase of this study demonstrated that the cannabis extracts significantly reduced spasticity and the frequency of spasms while improving sleep quality over an extended 12-wk period, compared to placebo controls[19]. This has led to approval of the use of Sativex in the treatment of MS-related spasticity. These studies demonstrate the potential beneficial roles of cannabis use in MS patients, although in general its supply and use remains illegal.

## ENDOCANNABINOID SYSTEM

The discovery of the endogenous cannabinoid (endocannabinoid) system that naturally exists in the body and responds to cannabinoids to exert their effects has aided research into the therapeutic utility of cannabinoids. This has fuelled the search for alternative modes of delivery into the human body, rather than the traditional method of "smoking". The endocannabinoid system consists of at least two families of lipid signalling molecules (the N-acyl ethanolamines and the monoacyl-glycerols), multiple enzymes in the biosynthesis and degradation of these lipids, as well as two G-protein coupled receptors [cannabinoid receptor type-1 ($CB_1$) and $CB_2$] (reviewed by[10]). $CB_1$ is mainly expressed in the CNS while $CB_2$ is predominantly found in leukocytes, while an increasing number of potential ligands and endocannabinoid degradation molecules are being isolated[20]. Several studies have highlighted the involvement of this system in regulating neurotransmission and its ability to prevent excessive neurotransmitter release, consistent with a capacity to provide symptomatic relief in MS[21,22].

Endocannabinoids are produced on demand and are retrogradely transported across the postsynaptic membrane to engage with $CB_1$ receptors, suppressing neurotransmitter release. The pharmacology of endocannabinoids is rapidly evolving with the discovery of new cannabinoid mimetics and novel CB receptor interactions that include orphan receptors and other CB receptors being proposed[23-26]. All these receptor functions are either sensitive to, or are regulated by CB receptors and culminate in neuropathic pain and inflammation, suggesting that novel drug targets based around these proteins might be useful in treating neuroinflammation and neuropathic pain in different neurological conditions. In addition, activation of the CB receptor inhibits adenylate cyclase that then reduces the levels of the second messenger cyclic adenosine monophosphate (cAMP), thus regulating cellular mechanisms such as cell fate[27]. CB receptor activation also inhibits voltage-dependent $Ca^{2+}$ channels and activates inwardly rectifying $K^+$ channels, a process that underlies CB-induced depression of excitatory neurotransmission[28,29]. Thus, the CB system is a potentially useful target for exploitation in neurodegenerative diseases such as MS.

## AXONAL DAMAGE IN MS

Although MS is defined as an inflammatory demyelinating disease of the CNS, axonal loss is also a key feature of the disease. In MS patients, analysis of their spinal cord lesions suggested that the permanent loss in function was not primarily due to demyelination but due to axonal loss[30]. Axonal loss is generally associated with inflammatory macrophage infiltration but is variable in MS lesions and can be severe in certain cases. For example, axonal density is reduced by 60%-70% in actively demyelinating lesions and is characterised by the presence of axonal spheroids, endbulbs, or focal accumulation of proteins[31-33]. Although shadow plaques appear after injury that is consistent with an attempt to remyelinate axons, ongoing axonal injury is present[34]. This suggests that during the early stages of remyelination axons are more susceptible to damage, a process that is related to the patterns of $Na^+$ channels at the widened surfaces of the nodes of Ranvier[35]. Axonal injury is also present in normal appearing white matter[36,37] and may occur as a result of secondary Wallerian degeneration[38]. However, this is not the only mechanism of axonal damage since two distinct patterns are observed: one that takes place within demyelinated lesions and correlates with lesional activity, while the other is diffuse axonal injury that is associated with inflammation and can additionally affect non-demyelinated nerve fibres[33].

Axonal injury in MS is also selective to the size of axon fibres. Small calibre axons are more prone to injury compared to thick axons and may relate to thin axons requiring a higher energy demand in terms of their critical mass of mitochondria[39]. Like MS, widespread axonal damage is also seen in experimental allergic encephalomyelitis (EAE)[34,40]. However, experimental models of MS reveal different mechanisms of axonal injury and there is currently no agreement for which mechanism is relevant to MS patients. Axonal injury mechanisms may involve T-lymphocytes and antibodies as part of the adaptive immune response as well as components of the innate immune system, driven by macrophages and microglia. For example, axonal injury can be driven by an antigen-specific cytotoxic T cell response, induced in neurons and glia by the expression of pro-inflammatory cytokines, leading to neuronal death and axonal transection[41-43].

Another mechanism of axonal damage relies on the production of auto-antibodies against cell surface molecules in neurons and axons. A subset of MS patients were described that mounted an antibody response against neurofascin, which is expressed on axons and oligodendrocyte processes at the nodes of Ranvier[44]. Systemic injection of neurofascin during EAE exacerbates the clinical symptoms of the disease with severe levels of axonal injury within lesions[44]. These observations suggest that auto-antibodies can directly mediate axonal damage. In MS lesions, axon damage is closely linked to

the presence of macrophages and microglia that are in intimate contact with axons[3,5] and are known to produce a number of cytotoxic molecules including reactive oxygen and nitric oxide intermediates[33]. The expression of these molecules, especially inducible nitric oxide synthase from macrophages, correlates with areas of axonal injury in acute EAE[45]. In summary, axonal damage is a feature of MS and EAE and correlates with functional deficits. EAE models demonstrate that axonal damage occurs through a variety of mechanisms. However, recent reports have highlighted the role of mitochondrial injury and subsequent energy failure, induced by oxygen and nitric oxide free radicals as a possible mechanism of axonal injury. Understanding of the pathway to axon injury will aid in the discovery of new molecules for therapeutic intervention.

## CANNABINOIDS IN MS

There is an abundance of evidence suggesting that MS patients gain symptomatic relief from cannabis extracts[46]. For example, Sativex, an oral-mucosal spray containing $\Delta^9$-THC and CBD is anti-spasmodic and analgesic in MS patients[47], while neuropathic pain associated with MS is relieved by dronabinol, an oral preparation of $\Delta^9$-THC analog[15]. Meta-analysis has also revealed that CB-based preparations are superior in the treatment of MS-related neuropathic pain than the placebo, confirming their beneficial effects in symptomatic relief[48]. This is consistent with the animal model of MS, EAE where treatment with exogenous cannabinoids controlled spasticity in chronic relapsing EAE models[49].

Modulation of the endocannabinoid system is also apparent in MS and EAE such that brain levels of CB receptors are downregulated in EAE while plasma levels of endocannabinoids are increased in MS patients[50,51]. Synthetic CB, HU-211, reduced the clinical severity of acute EAE in female Lewis rats as well as reducing inflammatory cell infiltration into the CNS[52], while the WIN55, 212-2, a $CB_1$ and $CB_2$ receptor agonist reduces T cell differentiation and hence reduces EAE severity in Theiler's murine encephalomyelitis virus-induced demyelinating disease, a mouse model of chronic-progressive MS[53], suggesting a key involvement of the CB receptors in the pathogenesis of EAE. Induction of EAE in $CB_1$ receptor-deficient mice causes rapid and progressively more neurodegeneration than in wild-type counterparts[54].

In our highly cited study on the role of cannabinoids in EAE, reported that the cannabinoid system was neuroprotective during EAE since $CB_1$-deficient mice poorly tolerated inflammatory and excitotoxic insults and showed significant accumulation of neurodegeneration after EAE[55]. We induced chronic relapsing EAE (CREAE) in wild type ABH, $CB_1$ gene (*Cnr1*)-deficient and congenic ABH. *Cnr1*$^{+/+}$ mice with mouse spinal cord homogenate emulsified in complete Freund's adjuvant on days 0 and 7 and monitored clinical disease progression over time. Mice developed characteristic paralytic disease episodes followed by remission with an increasing amount of residual deficit[49,56]. Whilst disease induction in both $CB_1$-deficient and $CB_1$ wild-type mice were similar, $CB_1$-deficient mice exhibited significantly higher levels of residual deficit. This deficit was quantitated in an open-field activity chamber and confirmed that $CB_1$-deficient mice displayed significantly more immobility and paresis than wild type mice, accumulating significantly more axonal damage after relapses. $CB_1$-deificent mice also developed spasticity after only a single attack, which is not seen in wild type mice until after three to four relapses of disease[49].

Numerous mechanisms cause neuronal death and axonal damage in EAE, including the influx of toxic ions such as $Ca^{2+}$ and caspase-3-mediated apoptosis[57]. $CB_1$-deficient mice demonstrated significantly lower levels of active caspase-3 during acute EAE compared with wild type mice, while caspase-3 was detected in dying axons, consistent with that observed in MS[3]. These results suggested that the elevated neurodegeneration in $CB_1$-deficient mice may be due to caspase-3-mediated apoptosis and axonal damage and hence agonism of the $CB_1$ receptor pathway is neuroprotective and may control neurological symptoms such as tremor and spasticity[49].

We also investigated whether glutamate toxicity can be regulated by cannabinoids[55]. Glutamate excitotoxity causes neuronal damage in both MS and EAE. For example, the glutamate antagonist, amantadine, reduces the relapse rate in MS patients[58], while elevated glutamate levels have been observed in cerebrospinal fluid from MS patients[59]. In EAE, the enhanced levels of glutamate agonists may result in aberrant astrocyte function, since activated astrocytes normally regulate glutamate levels through enzymes such as glutamate dehydrogenase and glutamine synthetase, both of which are down-regulated during EAE[60,61]. The amount of CNS glutamate is also affected by abnormal changes in neuronal and glial glutamate transporters, all of which raise the levels of glutamate in the CNS during EAE and ultimately lead to the synthesis of mediators responsible for neuronal dysfunction[59,62-64]. We reported that after *in vitro* stimulation of N-methyl-D-aspartic (NMDA) receptors in cerebellar granule cells, there was significantly more neuronal $Ca^{2+}$ influx in $CB_1$-deficient mice than in wild type controls suggesting that the cannabinoid receptors may tonically regulate $Ca^{2+}$ influx. The NMDA receptor antagonist MK-801 took longer to reduce $Ca^{2+}$ back to basal levels in $CB_1$-deficient mice than in congenic controls while $CB_1$ agonism with CP55, 940 inhibited NMDA-induced $Ca^{2+}$ influx in wild type mice but had no effect on $CB_1$-deficient mice. These results suggest that $Ca^{2+}$ is not only dysregulated in the absence of $CB_1$ receptors but that postsynaptic control of NMDA-receptor activation is also compromised. These *in vitro* results were confirmed by *in vivo* injections of kainic acid, a specific agonist of the kainate receptor that mimics the effects of glutamate, since injection of kainic acid in $CB_1$-deficient mice induced seizures and mortality within 10 min, while no effect of kainic acid was observed in wild type or congenic wild type control mice despite using 50-fold higher doses.

Therefore, CB1-receptors clearly regulate ionotropic glutamate receptor activity, leading to enhanced susceptibility to excitotoxic damage in $CB_1$-deficient mice.

Furthermore, we reported that $CB_1$ agonists protected mice against the consequences of CNS inflammation in models of experimental allergic uveitis (EAU)[55]. EAU is an inflammatory disease of the eye and after sensitization with for example, interphotoreceptor retinal binding peptide in B10. RIII mice, the neuroretina is completely destroyed within 14-16 d[65]. $CB_1$ receptor agonism with R(+)-WIN-55,212-2 and $\Delta^9$-THC both significantly inhibited photoreceptor damage without affecting inflammatory infiltrates suggesting that agonism of $CB_1$ is neuroprotective. Taken together, the results of our study demonstrated that cannabinoids protect against the neurodegenerative events in models of MS and EAU.

## CANNABINOID-1 RECEPTOR-MEDIATED NEUROPROTECTION IN MODELS OF MS

A feature of MS is neuronal loss, and in MS patients, loss of spinal cord axons correlate with neurological disability together with reduced N-acetyl aspartate levels in chronic MS patients[32]. Although axonal loss occurs early during the progression of MS[66], once a threshold of 15%-35% axonal loss in mice has been reached, permanent disability results[67,68]. In $CB_1$-deficient mice, significant axonal loss is evident after a single acute attack of EAE, suggesting that the mere presence of $CB_1$ is itself neuroprotective[55]. In EAE and MS, the presence of axonal damage correlates with inflammation that produces a range of neurotoxic agents such as glutamate, cytokines as well as creating oxidative stress in the environment of the CNS and damaging the blood-brain barrier[3,5,52,69-72].

Cannabinoids, however, can protect against acute hypoxia, excitotoxicity, oxidative and traumatic insults both *in vitro* and *in vivo*[73-76]. Cannabinoids can also inhibit both pre- and post-synaptic glutamate induced calcium responses and thus inhibit neurotoxicity[21,55,76], an effect that we showed to be $CB_1$-dependent[55]. In accord with our observations, $CB_1$-deficient mice were more susceptible to NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainite glutamate receptor excitotoxicity[55], however, $\Delta^9$-THC and CBD protected against NMDA-, AMPA- and kainite-agonist-induced cell death[77,78]. Delta-9-THC also protected retinal neurons from death induced by peroxynitrite-mediated NMDA-induced toxicity[79]. These observations demonstrate a clear neuroprotective role of cannabinoids in MS.

## CANNABINOID-2-MEDIATED EFFECTS IN MODELS OF MS

Although much of the focus in MS is devoted to $CB_1$ receptor pharmacology, the recognition that $CB_2$ receptors possess immunomodulatory properties has led to an increased focus on $CB_2$ receptors as potential therapeutic targets. Unlike $CB_1$ receptors, activation of the $CB_2$ receptor is not psychoactive and therefore targeting $CB_2$ receptors with selective agonists is a promising therapeutic avenue that is immunomodulatory without being psychoactive. For example, early indications came from experiments that showed that the administration of WIN55212-2, which functions as a $CB_1$ and $CB_2$ receptor agonist, attenuated the progression of EAE in C57BL/6 mice immunized with myelin oligodendrocyte-derived glycoprotein35-55 (MOG35-55)[80]. Furthermore, a selective antagonist of the $CB_1$ did not modulate the protective effect of WIN55212-2 but a selective antagonist of the $CB_2$ receptor blocked the effects of WIN55212-2. This led to the suggestion that the protective effects of WIN55212-2 was mediated through the $CB_2$ receptor[80]. However, later studies showed that the $CB_1$ receptor might play a neuroprotective role in the latter stages of EAE[49,54].

Other highly selective $CB_2$ receptor agonists such as O-1966 are also useful in the fight against MS since they do not produce psychoactive effects, determined by its low affinity to $CB_1$ receptors[81]. Administration of O-1966 in a chronic (C57BL/6/MOG), relapsing-remitting and an adoptive transfer model attenuated disease progression and improved motor function[82]. In addition, encephalitogenic T cells derived from $CB_2$-deficient mice were shown to be more aggressive in terms of CNS infiltration and increased severity of EAE, despite displaying similar levels of proliferation, apoptosis and cytokine production in the spleen as wild-type T cells[83]. Moreover, treatment of mice with $CB_2$ receptor agonists attenuate white cell trafficking across the blood-brain barrier while dendritic cells differentiated in the presence of selective $CB_2$ receptor agonist and inhibited T cell proliferation and shifted cytokine responses from inflammatory to anti-inflammatory molecules[82]. Recently, it has been shown that high concentrations of IFN-γ disrupts $P2X_7$ purinergic receptor signalling and thus inhibit the neuroprotective effects of endogenous cannabinoids[84]. Therefore, inhibiting IFN-γ by exogenous $CB_2$ agonists represents an obvious therapy to prevent the disruption of $P2X_7$ signalling.

## VALUE OF ANIMAL MODELS IN THE STUDY OF MULTIPLE SCLEROSIS

The validity of EAE as a model of MS is a topic of active debate with some researchers contenting that it is unsuitable due to its inability to mimic some of the pathological, immunologic and chronic features of MS. For example, EAE is usually monophasic whereas MS displays chronic relapsing features. Histological and magnetic resonance imaging data demonstrate axonal and cortical damage in MS but not in some models of EAE[85]. The extravasation of red blood cells into the CNS of swiss jim lambert mice and Lewis rats with EAE are not typical of MS[86,87], while encephalitogenic regions associated with myelin basic protein (MBP) or proteolipid protein normally activate more $CD4^+$ than $CD8^+$ T cells

but in inflammatory MS, $CD8^+$ T cell predominate[88-90]. However, there are many other immunological differences between mouse and human MS and these must be overcome if greater levels of success in drug development for human MS are to be achieved[91].

Several other points are worth considering in terms of the use of EAE as an animal model: (1) EAE provides little insight into the progression of MS in terms of the small amounts of demyelinated axons in EAE compared to MS while mice with EAE rarely exhibit ongoing functional deterioration that MS patients often display[92,93]; (2) The use of C57BL/6 mice in EAE studies limits the investigation of the mechanism of relapsing-remitting forms that more commonly affect MS patients (Ransohoff *et al*[93], 2002); (3) Treatment with factors that exert neurobiological effects also impact on immune and inflammatory cells and thus making results difficult to interpret (Ransohoff *et al*[93], 2002); and (4) EAE is generally generated using antigens that affect $CD4^+$ T cells while $CD8^+$ T cells that predominate in MS lesions are overlooked[92,94]. Likewise, the role of B cells is largely neglected despite recent data that demonstrate their importance in the pathogenesis of MS[95,96].

In summary, EAE has a long history in the fight against MS, however, its predictive value for treatment efficacy is poor. Nevertheless, it is widely used as a first-line animal model of MS and has provided mechanistic insights into the neuroinflammatory aspects of MS.

## LIMITATIONS OF ANIMAL MODELS OF MS

One of the biggest limitations on the use of EAE as a model of MS is the fact that disease has to be induced with complete Freund's adjuvant and heat-inactivated *Mycobacterium tuberculosis* rather than mimicking a spontaneous disease like MS. This leaves very little room for disease pathways and fails to represent the complexity of disease inducing mechanisms in MS. Demyelination, a feature of MS is also not obvious in all EAE models while the time course for disease manifestation may be days, EAE more closely resembles post-infectious acute demyelinating events[97]. In contrast, MS develops over years with patients presenting with more protracted epitope spreading than that observed in EAE mice[98]. There are also many other immunological differences between EAE and MS that need consideration in the development of potential therapies for MS.

Therapeutic developments from EAE have translated poorly to human MS. For example, only a few molecules that showed efficacy in EAE have been successful in MS trials. One of these molecules is Glatiramer acetate, a synthetic amino acid copolymer originally designed to mimic encephalitogenic MBP, but instead suppresses EAE by other mechanisms and reduced MS relapses by 30%[99,100]. However, the efficacy of Glatiramer acetate has been questioned by a systematic Cochrane review which calls into question the use of Glatiramer acetate in MS[101]. Tysabri (Natalizumab) and Gilenya (fingolimod) are the only two other drugs that have been licence for use in human MS. Natalizumab binds to the α4 subunit of α4β1 and α4β7 integrins and blocks binding to their endothelial receptors (VCAP-1 and mucosal addressin-cell adhesion molecule 1, thereby attenuating inflammation and ongoing inflammation[102,103]. Fingolimod is a sphingosine-1-phosphate-receptor modulator that prevents egress of lymphocytes from lymph nodes and significantly improved relapse rates compared to placebo controls[104]. At present therefore, it remains unclear why pre-clinical EAE studies predict treatment efficacy in human MS so poorly, however, EAE is still an important first-line model system in the development of new treatments for MS.

## BETTER ANIMAL MODELS OF MULTIPLE SCLEROSIS

To make greater progress, better animals models of MS need to be considered when testing new drugs. One fact that to be borne in mind is the fact that MS is a heterogeneous disease in terms of genetics, environmental effects, disease course, pathological treatments and treatment responsiveness[105]. Currently the majority of experiments are performed in inbred strains while clinically, the molecular mechanisms that determine the efficacy during prolonged follow-up of hundreds of patients are far more complex. Thus, it is likely that genetic differences account for some of the inter-patient differences in clinical efficacy of various drugs. Improved animal models may take into account the therapeutic effect of drugs in more than one animal model, treatments to be instigated after disease onset, long-term disease periods, use spontaneous disease models and incorporate human risk. The use of partially humanized mouse models to address the genetic and disease variability are a step in the right direction towards developing better therapeutics for MS[106].

## SUMMARY AND FUTURE PROSPECTS

Studies have shown that antagonism of the $CB_1$ receptor pathway contributes to neuronal damage in CREAE and the relative worsening of tremor and spasticity. The addition of exogenous $CB_1$ agonists derived from cannabis afforded significant neuroprotection from the consequences of inflammatory CNS disease in EAE and EAU models. Although clear neuroprotective benefits of cannabinoids have been demonstrated, the unwanted psychotropic effects need to be addressed. The adverse psychotropic effects, its role in appetite, pain and cognition together with the observation that the $CB_1$ receptor is downregulated in some neurodegenerative diseases limits the usefulness of cannabinoids in the treatment of MS. Alternative treatments may be more useful and includes the development of drugs based on CBD, the non-psychoactive part of cannabis that possess anti-inflammatory and anti-oxidant properties. Furthermore,

the $CB_2$ receptor is being recognised as a potential target since it regulates neuroinflammation and neurogenesis while being non-psychoactive.

In summary, the endocannabinoid system exerts multiple actions and may be useful in the development of therapies to treat neurodegenerative diseases. However, the biggest challenge remains, namely the development of drugs that lack the adverse psychoactive side effects.

## REFERENCES


1 **Compston A**, Coles A. Multiple sclerosis. *Lancet* 2002; **359**: 1221-1231 [PMID: 11955556 DOI: 10.1016/S0140-6736(02)08220-X]
2 **Compston A**, Coles A. Multiple sclerosis. *Lancet* 2008; **372**: 1502-1517 [PMID: 18970977 DOI: 10.1016/S0140-6736(08)61620-7]
3 **Trapp BD**, Peterson J, Ransohoff RM, Rudick R, Mörk S, Bö L. Axonal transection in the lesions of multiple sclerosis. *N Engl J Med* 1998; **338**: 278-285 [PMID: 9445407 DOI: 10.1056/NEJM199801293380502]
4 **Coles AJ**, Wing MG, Molyneux P, Paolillo A, Davie CM, Hale G, Miller D, Waldmann H, Compston A. Monoclonal antibody treatment exposes three mechanisms underlying the clinical course of multiple sclerosis. *Ann Neurol* 1999; **46**: 296-304 [PMID: 10482259]
5 **Ferguson B**, Matyszak MK, Esiri MM, Perry VH. Axonal damage in acute multiple sclerosis lesions. *Brain* 1997; **120** (Pt 3): 393-399 [PMID: 9126051]
6 **Bjartmar C**, Wujek JR, Trapp BD. Axonal loss in the pathology of MS: consequences for understanding the progressive phase of the disease. *J Neurol Sci* 2003; **206**: 165-171 [PMID: 12559505]
7 **Smith KJ**, Kapoor R, Hall SM, Davies M. Electrically active axons degenerate when exposed to nitric oxide. *Ann Neurol* 2001; **49**: 470-476 [PMID: 11310624]
8 **Chaturvedi GN**, Tiwari SK, Rai NP. Medicinal use of opium and cannabis in medieval India. *Indian J Hist Sci* 1981; **16**: 31-35 [PMID: 11611777]
9 **Pertwee RG**. Cannabinoids and multiple sclerosis. *Pharmacol Ther* 2002; **95**: 165-174 [PMID: 12182963]
10 **Rog DJ**. Cannabis-based medicines in multiple sclerosis--a review of clinical studies. *Immunobiology* 2010; **215**: 658-672 [PMID: 20541836 DOI: 10.1016/j.imbio.2010.03.009]
11 **Gaoni Y**, Mechoulam R. The isolation and structure of delta-1-tetrahydrocannabinol and other neutral cannabinoids from hashish. *J Am Chem Soc* 1971; **93**: 217-224 [PMID: 5538858]
12 **Gowran A**, Noonan J, Campbell VA. The multiplicity of action of cannabinoids: implications for treating neurodegeneration. *CNS Neurosci Ther* 2011; **17**: 637-644 [PMID: 20875047 DOI: 10.1111/j.1755-5949.2010.00195.x]
13 **Smith PF**. Cannabinoids for the treatment of multiple sclerosis: no smoke without fire? *Expert Rev Neurother* 2003; **3**: 327-334 [PMID: 19810900 DOI: 10.1586/14737175.3.3.327]
14 **Killestein J**, Hoogervorst EL, Reif M, Blauw B, Smits M, Uitdehaag BM, Nagelkerken L, Polman CH. Immunomodulatory effects of orally administered cannabinoids in multiple sclerosis. *J Neuroimmunol* 2003; **137**: 140-143 [PMID: 12667658]
15 **Zajicek J**, Fox P, Sanders H, Wright D, Vickery J, Nunn A, Thompson A. Cannabinoids for treatment of spasticity and other symptoms related to multiple sclerosis (CAMS study): multicentre randomised placebo-controlled trial. *Lancet* 2003; **362**: 1517-1526 [PMID: 14615106 DOI: 10.1016/S0140-6736(03)14738-1]
16 **Zajicek JP**, Sanders HP, Wright DE, Vickery PJ, Ingram WM, Reilly SM, Nunn AJ, Teare LJ, Fox PJ, Thompson AJ. Cannabinoids in multiple sclerosis (CAMS) study: safety and efficacy data for 12 months follow up. *J Neurol Neurosurg Psychiatry* 2005; **76**: 1664-1669 [PMID: 16291891 DOI: 10.1136/jnnp.2005.070136]
17 **Chong MS**, Wolff K, Wise K, Tanton C, Winstock A, Silber E. Cannabis use in patients with multiple sclerosis. *Mult Scler* 2006; **12**: 646-651 [PMID: 17086912]
18 **Consroe P**, Musty R, Rein J, Tillery W, Pertwee R. The perceived effects of smoked cannabis on patients with multiple sclerosis. *Eur Neurol* 1997; **38**: 44-48 [PMID: 9252798]
19 **Novotna A**, Mares J, Ratcliffe S, Novakova I, Vachova M, Zapletalova O, Gasperini C, Pozzilli C, Cefaro L, Comi G, Rossi P, Ambler Z, Stelmasiak Z, Erdmann A, Montalban X, Klimek A, Davies P. A randomized, double-blind, placebo-controlled, parallel-group, enriched-design study of nabiximols* (Sativex(®) ), as add-on therapy, in subjects with refractory spasticity caused by multiple sclerosis. *Eur J Neurol* 2011; **18**: 1122-1131 [PMID: 21362108 DOI: 10.1111/j.1468-1331.2010.03328.x]
20 **Howlett AC**, Barth F, Bonner TI, Cabral G, Casellas P, Devane WA, Felder CC, Herkenham M, Mackie K, Martin BR, Mechoulam R, Pertwee RG. International Union of Pharmacology. XXVII. Classification of cannabinoid receptors. *Pharmacol Rev* 2002; **54**: 161-202 [PMID: 12037135]
21 **Wilson RI**, Nicoll RA. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature* 2001; **410**: 588-592 [PMID: 11279497 DOI: 10.1038/35069076]
22 **Kreitzer AC**, Carter AG, Regehr WG. Inhibition of interneuron firing extends the spread of endocannabinoid signaling in the cerebellum. *Neuron* 2002; **34**: 787-796 [PMID: 12062024]
23 **Thakur GA**, Tichkule R, Bajaj S, Makriyannis A. Latest advances in cannabinoid receptor agonists. *Expert Opin Ther Pat* 2009; **19**: 1647-1673 [PMID: 19939187 DOI: 10.1517/13543770903436505]
24 **Brown AJ**. Novel cannabinoid receptors. *Br J Pharmacol* 2007; **152**: 567-575 [PMID: 17906678 DOI: 10.1038/sj.bjp.0707481]
25 **Sharir H**, Abood ME. Pharmacological characterization of GPR55, a putative cannabinoid receptor. *Pharmacol Ther* 2010; **126**: 301-313 [PMID: 20298715 DOI: 10.1016/j.pharmthera.2010.02.004]
26 **Nevalainen T**, Irving AJ. GPR55, a lysophosphatidylinositol receptor with cannabinoid sensitivity? *Curr Top Med Chem* 2010; **10**: 799-813 [PMID: 20370712]
27 **Miyamoto N**, Tanaka R, Zhang N, Shimura H, Onodera M, Mochizuki H, Hattori N, Urabe T. Crucial role for Ser133-phosphorylated form of cyclic AMP-responsive element binding protein signaling in the differentiation and survival of neural progenitors under chronic cerebral hypoperfusion. *Neuroscience* 2009; **162**: 525-536 [PMID: 19426786 DOI: 10.1016/j.neuroscience.2009.05.004]
28 **Hampson AJ**, Grimaldi M, Lolic M, Wink D, Rosenthal R, Axelrod J. Neuroprotective antioxidants from marijuana. *Ann N Y Acad Sci* 2000; **899**: 274-282 [PMID: 10863546]
29 **Nicholson RA**, Liao C, Zheng J, David LS, Coyne L, Errington AC, Singh G, Lees G. Sodium channel inhibition by anandamide and synthetic cannabimimetics in brain. *Brain Res* 2003; **978**: 194-204 [PMID: 12834914]
30 **Kornek B**, Lassmann H. Axonal pathology in multiple sclerosis. A historical note. *Brain Pathol* 1999; **9**: 651-656 [PMID: 10517504]
31 **Mews I**, Bergmann M, Bunkowski S, Gullotta F, Brück W. Oligodendrocyte and axon pathology in clinically silent multiple sclerosis lesions. *Mult Scler* 1998; **4**: 55-62 [PMID: 9599334]
32 **Bjartmar C**, Kidd G, Mörk S, Rudick R, Trapp BD. Neurological disability correlates with spinal cord axonal loss and reduced N-acetyl aspartate in chronic multiple sclerosis patients. *Ann Neurol* 2000; **48**: 893-901 [PMID: 11117546]
33 **Lassmann H**. Axonal and neuronal pathology in multiple sclerosis: what have we learnt from animal models. *Exp Neurol* 2010; **225**: 2-8 [PMID: 19840788 DOI: 10.1016/j.expneurol.2009.10.009]
34 **Kornek B**, Storch MK, Weissert R, Wallstroem E, Stefferl A, Olsson T, Linington C, Schmidbauer M, Lassmann H. Multiple sclerosis and chronic autoimmune encephalomyelitis: a comparative quantitative study of axonal

injury in active, inactive, and remyelinated lesions. *Am J Pathol* 2000; **157**: 267-276 [PMID: 10880396 DOI: 10.1016/S0002-9440(10)64537-3]
35 **Smith KJ**. Axonal protection in multiple sclerosis--a particular need during remyelination? *Brain* 2006; **129**: 3147-3149 [PMID: 17132643 DOI: 10.1093/brain/awl323]
36 **Kutzelnigg A**, Lucchinetti CF, Stadelmann C, Brück W, Rauschka H, Bergmann M, Schmidbauer M, Parisi JE, Lassmann H. Cortical demyelination and diffuse white matter injury in multiple sclerosis. *Brain* 2005; **128**: 2705-2712 [PMID: 16230320 DOI: 10.1093/brain/awh641]
37 **Frischer JM**, Bramow S, Dal-Bianco A, Lucchinetti CF, Rauschka H, Schmidbauer M, Laursen H, Sorensen PS, Lassmann H. The relation between inflammation and neurodegeneration in multiple sclerosis brains. *Brain* 2009; **132**: 1175-1189 [PMID: 19339255 DOI: 10.1093/brain/awp070]
38 **Evangelou N**, Konz D, Esiri MM, Smith S, Palace J, Matthews PM. Regional axonal loss in the corpus callosum correlates with cerebral white matter lesion volume and distribution in multiple sclerosis. *Brain* 2000; **123** (Pt 9): 1845-1849 [PMID: 10960048]
39 **Evangelou N**, Konz D, Esiri MM, Smith S, Palace J, Matthews PM. Size-selective neuronal changes in the anterior optic pathways suggest a differential susceptibility to injury in multiple sclerosis. *Brain* 2001; **124**: 1813-1820 [PMID: 11522583]
40 **Linington C**, Bradl M, Lassmann H, Brunner C, Vass K. Augmentation of demyelination in rat acute allergic encephalomyelitis by circulating mouse monoclonal antibodies directed against a myelin/oligodendrocyte glycoprotein. *Am J Pathol* 1988; **130**: 443-454 [PMID: 2450462]
41 **Neumann H**, Cavalié A, Jenne DE, Wekerle H. Induction of MHC class I genes in neurons. *Science* 1995; **269**: 549-552 [PMID: 7624779]
42 **Medana IM**, Gallimore A, Oxenius A, Martinic MM, Wekerle H, Neumann H. MHC class I-restricted killing of neurons by virus-specific CD8+ T lymphocytes is effected through the Fas/FasL, but not the perforin pathway,. *Eur J Immunol* 2000; **30**: 3623-3633 [PMID: 11169405 DOI: 10.1002/1521-4141(200012)30: 12< 3623: : AID-IMMU3623> 3.0.CO; 2-F]
43 **Medana I**, Martinic MA, Wekerle H, Neumann H. Transection of major histocompatibility complex class I-induced neurites by cytotoxic T lymphocytes. *Am J Pathol* 2001; **159**: 809-815 [PMID: 11549572 DOI: 10.1016/S0002-9440(10)61755-5]
44 **Mathey EK**, Derfuss T, Storch MK, Williams KR, Hales K, Woolley DR, Al-Hayani A, Davies SN, Rasband MN, Olsson T, Moldenhauer A, Velhin S, Hohlfeld R, Meinl E, Linington C. Neurofascin as a novel target for autoantibody-mediated axonal injury. *J Exp Med* 2007; **204**: 2363-2372 [PMID: 17846150 DOI: 10.1084/jem.20071053]
45 **Aboul-Enein F**, Weiser P, Höftberger R, Lassmann H, Bradl M. Transient axonal injury in the absence of demyelination: a correlate of clinical disease in acute experimental autoimmune encephalomyelitis. *Acta Neuropathol* 2006; **111**: 539-547 [PMID: 16718350 DOI: 10.1007/s00401-006-0047-y]
46 **Lakhan SE**, Rowland M. Whole plant cannabis extracts in the treatment of spasticity in multiple sclerosis: a systematic review. *BMC Neurol* 2009; **9**: 59 [PMID: 19961570 DOI: 10.1186/1471-2377-9-59]
47 **Rog DJ**, Nurmikko TJ, Young CA. Oromucosal delta9-tetrahydrocannabinol/cannabidiol for neuropathic pain associated with multiple sclerosis: an uncontrolled, open-label, 2-year extension trial. *Clin Ther* 2007; **29**: 2068-2079 [PMID: 18035205 DOI: 10.1016/j.clinthera.2007.09.013]
48 **Iskedjian M**, Bereza B, Gordon A, Piwko C, Einarson TR. Meta-analysis of cannabis based treatments for neuropathic and multiple sclerosis-related pain. *Curr Med Res Opin* 2007; **23**: 17-24 [PMID: 17257464 DOI: 10.1185/030079906X158066]
49 **Baker AM**, Grekova MC, Richert JR. EAE susceptibility in FVB mice. *J Neurosci Res* 2000; **61**: 140-145 [PMID: 10878586]
50 **Jean-Gilles L**, Feng S, Tench CR, Chapman V, Kendall DA, Barrett DA, Constantinescu CS. Plasma endocannabinoid levels in multiple sclerosis. *J Neurol Sci* 2009; **287**: 212-215 [PMID: 19695579 DOI: 10.1016/j.jns.2009.07.021]
51 **Centonze D**, Bari M, Rossi S, Prosperetti C, Furlan R, Fezza F, De Chiara V, Battistini L, Bernardi G, Bernardini S, Martino G, Maccarrone M. The endocannabinoid system is dysregulated in multiple sclerosis and in experimental autoimmune encephalomyelitis. *Brain* 2007; **130**: 2543-2553 [PMID: 17626034 DOI: 10.1093/brain/awm160]
52 **Achiron A**, Miron S, Lavie V, Margalit R, Biegon A. Dexanabinol (HU-211) effect on experimental autoimmune encephalomyelitis: implications for the treatment of acute relapses of multiple sclerosis. *J Neuroimmunol* 2000; **102**: 26-31 [PMID: 10626663]
53 **Croxford JL**. Therapeutic potential of cannabinoids in CNS disease. *CNS Drugs* 2003; **17**: 179-202 [PMID: 12617697]
54 **Maresz K**, Pryce G, Ponomarev ED, Marsicano G, Croxford JL, Shriver LP, Ledent C, Cheng X, Carrier EJ, Mann MK, Giovannoni G, Pertwee RG, Yamamura T, Buckley NE, Hillard CJ, Lutz B, Baker D, Dittel BN. Direct suppression of CNS autoimmune inflammation via the cannabinoid receptor CB1 on neurons and CB2 on autoreactive T cells. *Nat Med* 2007; **13**: 492-497 [PMID: 17401376 DOI: 10.1038/nm1561]
55 **Pryce G**, Ahmed Z, Hankey DJ, Jackson SJ, Croxford JL, Pocock JM, Ledent C, Petzold A, Thompson AJ, Giovannoni G, Cuzner ML, Baker D. Cannabinoids inhibit neurodegeneration in models of multiple sclerosis. *Brain* 2003; **126**: 2191-2202 [PMID: 12876144 DOI: 10.1093/brain/awg224]
56 **Baker D**, O'Neill JK, Gschmeissner SE, Wilcox CE, Butter C, Turk JL. Induction of chronic relapsing experimental allergic encephalomyelitis in Biozzi mice. *J Neuroimmunol* 1990; **28**: 261-270 [PMID: 2373763]
57 **Ahmed Z**, Doward AI, Pryce G, Taylor DL, Pocock JM, Leonard JP, Baker D, Cuzner ML. A role for caspase-1 and -3 in the pathology of experimental allergic encephalomyelitis : inflammation versus degeneration. *Am J Pathol* 2002; **161**: 1577-1586 [PMID: 12414506 DOI: 10.1016/S0002-9440(10)64436-7]
58 **Plaut GS**. Effectiveness of amantadine in reducing relapses in multiple sclerosis. *J R Soc Med* 1987; **80**: 91-93 [PMID: 3550084]
59 **Stover JF**, Pleines UE, Morganti-Kossmann MC, Kossmann T, Lowitzsch K, Kempski OS. Neurotransmitters in cerebrospinal fluid reflect pathological activity. *Eur J Clin Invest* 1997; **27**: 1038-1043 [PMID: 9466133]
60 **Hardin-Pouzet H**, Krakowski M, Bourbonnière L, Didier-Bazes M, Tran E, Owens T. Glutamate metabolism is downregulated in astrocytes during experimental allergic encephalomyelitis. *Glia* 1997; **20**: 79-85 [PMID: 9145307]
61 **Rothstein JD**. Neurobiology. Bundling up excitement. *Nature* 2000; **407**: 141, 143 [PMID: 11001037 DOI: 10.1038/35025170]
62 **Ohgoh M**, Hanada T, Smith T, Hashimoto T, Ueno M, Yamanishi Y, Watanabe M, Nishizawa Y. Altered expression of glutamate transporters in experimental autoimmune encephalomyelitis. *J Neuroimmunol* 2002; **125**: 170-178 [PMID: 11960654]
63 **Piani D**, Frei K, Do KQ, Cuénod M, Fontana A. Murine brain macrophages induced NMDA receptor mediated neurotoxicity in vitro by secreting glutamate. *Neurosci Lett* 1991; **133**: 159-162 [PMID: 1687755]
64 **Smith QR**. Transport of glutamate and other amino acids at the blood-brain barrier. *J Nutr* 2000; **130**: 1016S-1022S [PMID: 10736373]
65 **Hankey DJ**, Nickerson JM, Donoso LA, Lightman SL, Baker D. Experimental autoimmune uveoretinitis in mice (Biozzi ABH and NOD) expressing the autoimmune-associated H-2A(g7) molecule: identification of a uveitogenic epitope. *J Neuroimmunol* 2001; **118**: 212-222 [PMID: 11498256]
66 **Filippi M**, Rocca MA. MRI aspects of the "inflammatory phase" of multiple sclerosis. *Neurol Sci* 2003; **24** Suppl 5: S275-S278 [PMID: 14652788 DOI: 10.1007/s10072-003-0173-4]
67 **Confavreux C**, Vukusic S, Moreau T, Adeleine P. Relapses

and progression of disability in multiple sclerosis. *N Engl J Med* 2000; **343**: 1430-1438 [PMID: 11078767 DOI: 10.1056/NEJM200011163432001]

68 **Wujek JR**, Bjartmar C, Richer E, Ransohoff RM, Yu M, Tuohy VK, Trapp BD. Axon loss in the spinal cord determines permanent neurological disability in an animal model of multiple sclerosis. *J Neuropathol Exp Neurol* 2002; **61**: 23-32 [PMID: 11829341]

69 **Bolton C**, Paul C. MK-801 limits neurovascular dysfunction during experimental allergic encephalomyelitis. *J Pharmacol Exp Ther* 1997; **282**: 397-402 [PMID: 9223580]

70 **Pitt D**, Werner P, Raine CS. Glutamate excitotoxicity in a model of multiple sclerosis. *Nat Med* 2000; **6**: 67-70 [PMID: 10613826 DOI: 10.1038/71555]

71 **Koprowski H**, Zheng YM, Heber-Katz E, Fraser N, Rorke L, Fu ZF, Hanlon C, Dietzschold B. In vivo expression of inducible nitric oxide synthase in experimentally induced neurologic diseases. *Proc Natl Acad Sci USA* 1993; **90**: 3024-3027 [PMID: 7681993]

72 **Lock C**, Hermans G, Pedotti R, Brendolan A, Schadt E, Garren H, Langer-Gould A, Strober S, Cannella B, Allard J, Klonowski P, Austin A, Lad N, Kaminski N, Galli SJ, Oksenberg JR, Raine CS, Heller R, Steinman L. Gene-microarray analysis of multiple sclerosis lesions yields new targets validated in autoimmune encephalomyelitis. *Nat Med* 2002; **8**: 500-508 [PMID: 11984595 DOI: 10.1038/nm0502-500]

73 **Shen M**, Thayer SA. Cannabinoid receptor agonists protect cultured rat hippocampal neurons from excitotoxicity. *Mol Pharmacol* 1998; **54**: 459-462 [PMID: 9730904]

74 **Nagayama T**, Sinor AD, Simon RP, Chen J, Graham SH, Jin K, Greenberg DA. Cannabinoids and neuroprotection in global and focal cerebral ischemia and in neuronal cultures. *J Neurosci* 1999; **19**: 2987-2995 [PMID: 10191316]

75 **Sinor AD**, Irvin SM, Greenberg DA. Endocannabinoids protect cerebral cortical neurons from in vitro ischemia in rats. *Neurosci Lett* 2000; **278**: 157-160 [PMID: 10653017]

76 **Abood ME**, Rizvi G, Sallapudi N, McAllister SD. Activation of the CB1 cannabinoid receptor protects cultured mouse spinal neurons against excitotoxicity. *Neurosci Lett* 2001; **309**: 197-201 [PMID: 11514075]

77 **Hampson AJ**, Grimaldi M, Axelrod J, Wink D. Cannabidiol and (-)Delta9-tetrahydrocannabinol are neuroprotective antioxidants. *Proc Natl Acad Sci USA* 1998; **95**: 8268-8273 [PMID: 9653176]

78 **Marsicano G**, Moosmann B, Hermann H, Lutz B, Behl C. Neuroprotective properties of cannabinoids against oxidative stress: role of the cannabinoid receptor CB1. *J Neurochem* 2002; **80**: 448-456 [PMID: 11905991]

79 **El-Remessy AB**, Khalil IE, Matragoon S, Abou-Mohamed G, Tsai NJ, Roon P, Caldwell RB, Caldwell RW, Green K, Liou GI. Neuroprotective effect of (-)Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. *Am J Pathol* 2003; **163**: 1997-2008 [PMID: 14578199]

80 **Ni X**, Geller EB, Eppihimer MJ, Eisenstein TK, Adler MW, Tuma RF. Win 55212-2, a cannabinoid receptor agonist, attenuates leukocyte/endothelial interactions in an experimental autoimmune encephalomyelitis model. *Mult Scler* 2004; **10**: 158-164 [PMID: 15124761]

81 **Wiley JL**, Beletskaya ID, Ng EW, Dai Z, Crocker PJ, Mahadevan A, Razdan RK, Martin BR. Resorcinol derivatives: a novel template for the development of cannabinoid CB(1)/CB(2) and CB(2)-selective agonists. *J Pharmacol Exp Ther* 2002; **301**: 679-689 [PMID: 11961073]

82 **Zhang M**, Martin BR, Adler MW, Razdan RJ, Kong W, Ganea D, Tuma RF. Modulation of cannabinoid receptor activation as a neuroprotective strategy for EAE and stroke. *J Neuroimmune Pharmacol* 2009; **4**: 249-259 [PMID: 19255856 DOI: 10.1007/s11481-009-9148-4]

83 **Dittel BN**. Direct suppression of autoreactive lymphocytes in the central nervous system via the CB2 receptor. *Br J Pharmacol* 2008; **153**: 271-276 [PMID: 17922025 DOI: 10.1038/sj.bjp.0707493]

84 **Whiting P**, Harbord R, Main C, Deeks JJ, Filippini G, Egger M, Sterne JA. Accuracy of magnetic resonance imaging for the diagnosis of multiple sclerosis: systematic review. *BMJ* 2006; **332**: 875-884 [PMID: 16565096 DOI: 10.1136/bmj.38771.583796.7C]

85 **Sriram S**, Yao SY, Stratton C, Moses H, Narayana PA, Wolinsky JS. Pilot study to examine the effect of antibiotic therapy on MRI outcomes in RRMS. *J Neurol Sci* 2005; **234**: 87-91 [PMID: 15935383 DOI: 10.1016/j.jns.2005.03.042]

86 **Raine CS**, Traugott U, Nussenblatt RB, Stone SH. Optic neuritis and chronic relapsing experimental allergic encephalomyelitis: relationship to clinical course and comparison with multiple sclerosis. *Lab Invest* 1980; **42**: 327-335 [PMID: 7189001]

87 **Forge JK**, Pedchenko TV, LeVine SM. Iron deposits in the central nervous system of SJL mice with experimental allergic encephalomyelitis. *Life Sci* 1998; **63**: 2271-2284 [PMID: 9870713]

88 **Booss J**, Esiri MM, Tourtellotte WW, Mason DY. Immunohistological analysis of T lymphocyte subsets in the central nervous system in chronic progressive multiple sclerosis. *J Neurol Sci* 1983; **62**: 219-232 [PMID: 6607973]

89 **Hauser SL**, Bhan AK, Gilles F, Kemp M, Kerr C, Weiner HL. Immunohistochemical analysis of the cellular infiltrate in multiple sclerosis lesions. *Ann Neurol* 1986; **19**: 578-587 [PMID: 3524414 DOI: 10.1002/ana.410190610]

90 **Lassmann H**, Ransohoff RM. The CD4-Th1 model for multiple sclerosis: a critical [correction of crucial] re-appraisal. *Trends Immunol* 2004; **25**: 132-137 [PMID: 15036040 DOI: 10.1016/j.it.2004.01.007]

91 **Friese MA**, Montalban X, Willcox N, Bell JI, Martin R, Fugger L. The value of animal models for drug development in multiple sclerosis. *Brain* 2006; **129**: 1940-1952 [PMID: 16636022 DOI: 10.1093/brain/awl083]

92 **Ransohoff RM**. Animal models of multiple sclerosis: the good, the bad and the bottom line. *Nat Neurosci* 2012; **15**: 1074-1077 [PMID: 22837037 DOI: 10.1038/nn.3168]

93 **Ransohoff RM**, Howe CL, Rodriguez M. Growth factor treatment of demyelinating disease: at last, a leap into the light. *Trends Immunol* 2002; **23**: 512-516 [PMID: 12401395]

94 **Huseby ES**, Liggitt D, Brabb T, Schnabel B, Ohlén C, Goverman J. A pathogenic role for myelin-specific CD8(+) T cells in a model for multiple sclerosis. *J Exp Med* 2001; **194**: 669-676 [PMID: 11535634]

95 **Hauser SL**, Waubant E, Arnold DL, Vollmer T, Antel J, Fox RJ, Bar-Or A, Panzara M, Sarkar N, Agarwal S, Langer-Gould A, Smith CH. B-cell depletion with rituximab in relapsing-remitting multiple sclerosis. *N Engl J Med* 2008; **358**: 676-688 [PMID: 18272891 DOI: 10.1056/NEJMoa0706383]

96 **Berer K**, Wekerle H, Krishnamoorthy G. B cells in spontaneous autoimmune diseases of the central nervous system. *Mol Immunol* 2011; **48**: 1332-1337 [PMID: 21146219 DOI: 10.1016/j.molimm.2010.10.025]

97 **Steinman L**. Assessment of animal models for MS and demyelinating disease in the design of rational therapy. *Neuron* 1999; **24**: 511-514 [PMID: 10595504]

98 **Vanderlugt CL**, Miller SD. Epitope spreading in immune-mediated diseases: implications for immunotherapy. *Nat Rev Immunol* 2002; **2**: 85-95 [PMID: 11910899 DOI: 10.1038/nri724]

99 **Teitelbaum D**, Meshorer A, Hirshfeld T, Arnon R, Sela M. Suppression of experimental allergic encephalomyelitis by a synthetic polypeptide. *Eur J Immunol* 1971; **1**: 242-248 [PMID: 5157960 DOI: 10.1002/eji.1830010406]

100 **Johnson KP**, Brooks BR, Cohen JA, Ford CC, Goldstein J, Lisak RP, Myers LW, Panitch HS, Rose JW, Schiffer RB. Copolymer 1 reduces relapse rate and improves disability in relapsing-remitting multiple sclerosis: results of a phase III multicenter, double-blind placebo-controlled trial. The Co-

polymer 1 Multiple Sclerosis Study Group. *Neurology* 1995; **45**: 1268-1276 [PMID: 7617181]

101 **Munari L**, Lovati R, Boiko A. Therapy with glatiramer acetate for multiple sclerosis. *Cochrane Database Syst Rev* 2004; (**1**): CD004678 [PMID: 14974077 DOI: 10.1002/14651858.CD004678]

102 **Yednock TA**, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against alpha 4 beta 1 integrin. *Nature* 1992; **356**: 63-66 [PMID: 1538783 DOI: 10.1038/356063a0]

103 **Polman CH**, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, Phillips JT, Lublin FD, Giovannoni G, Wajgt A, Toal M, Lynn F, Panzara MA, Sandrock AW. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. *N Engl J Med* 2006; **354**: 899-910 [PMID: 16510744 DOI: 10.1056/NEJMoa044397]

104 **Kappos L**, Comi G, Panitch H, Oger J, Antel J, Conlon P, Steinman L. Induction of a non-encephalitogenic type 2 T helper-cell autoimmune response in multiple sclerosis after administration of an altered peptide ligand in a placebo-controlled, randomized phase II trial. The Altered Peptide Ligand in Relapsing MS Study Group. *Nat Med* 2000; **6**: 1176-1182 [PMID: 11017151 DOI: 10.1038/80525]

105 **Lassmann H**, Brück W, Lucchinetti C. Heterogeneity of multiple sclerosis pathogenesis: implications for diagnosis and therapy. *Trends Mol Med* 2001; **7**: 115-121 [PMID: 11286782]

106 **Gregersen JW**, Holmes S, Fugger L. Humanized animal models for autoimmune diseases. *Tissue Antigens* 2004; **63**: 383-394 [PMID: 15104670 DOI: 10.1111/j.0001-2815.2004.00243.x]

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REVIEW

# Secondary prevention of ischaemic stroke

Irene Volonghi, Alessandro Padovani, Elisabetta Del Zotto, Alessia Giossi, Paolo Costa, Andrea Morotti, Loris Poli, Alessandro Pezzini

Irene Volonghi, Alessandro Padovani, Paolo Costa, Andrea Morotti, Loris Poli, Alessandro Pezzini, Dipartimento di Scienze Cliniche e Sperimentali, Clinica Neurologica, Università degli Studi di Brescia, 25123 Brescia, Italy
Elisabetta Del Zotto, U.O. di Recupero e Rieducazione Funzionale, IRCCS Fondazione Don Gnocchi, 20149 Rovato (Brescia), Italy
Alessia Giossi, U.O. Neurologia, Istituto Clinico Sant'Anna, 25127 Brescia, Italy
Author contributions: Volonghi I and Pezzini A contributed to manuscript conception, data acquisition, revision, and wrote the paper; Padovani A contributed to manuscript conception, data acquisition and critical revision for important intellectual content; Del Zotto E, Giossi A, Costa P, Morotti A and Poli L contributed to manuscript conception and data acquisition; All the authors gave approval to final version of the manuscript.
Correspondence to: Irene Volonghi, MD, Dipartimento di Scienze Cliniche e Sperimentali, Clinica Neurologica, Università degli Studi di Brescia, P.le Spedali Civili 1, 25123 Brescia, Italy. irene.volonghi@hotmail.it
Telephone: +39-30-3384086 Fax: +39-30-3384086
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## Abstract

In spite of a documented reduction in incidence in high-income countries over the last decades, stroke is still a leading cause of death and disability worldwide. With the ageing of the population stroke-related economic burden is expected to increase, because of residual disability and its complications, such as cognitive impairment, high risk of falls and fractures, depression and epilepsy. Furthermore, because of the substantial rate of early and long-term vascular recurrences after the first event, secondary prevention after cerebral ischaemia is a crucial issue. This is even more important after minor stroke and transient ischaemic attack (TIA), in order to reduce the risk of potentially more severe and disabling events. To accomplish this aim, acute long-term medical and surgical treatments as well as lifestyle modifications are strongly recommended. However, apart from the well-established indications to thrombolysis, studies in acute phase after a first stroke or TIA are scarce and evidence is lacking. More trials are available for long-term secondary prevention with different classes of drugs, including antithrombotic medications for ischaemic events of arterial and cardiac origin, especially related to atrial fibrillation (antiplatelets and anticoagulants, respectively), lipid lowering agents (mainly statins), blood pressure lowering drugs, surgical and endovascular revascularization procedures.

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**Key words:** Stroke; Transient ischaemic attack; Secondary prevention; Antiplatelets; Anticoagulants; Medical stroke treatment; Carotid stenosis

**Core tip:** Aggressive and combination treatments in the acute phase after transient ischaemic attack or minor stroke have been shown to be beneficial in few studies, but results of ongoing randomized trials are required. On the other side, in long-term prevention the most important innovation is the advent of new anticoagulant agents for stroke prevention in atrial fibrillation. Recent trials showed efficacy and safety of thrombin and factor Xa inhibitors, compared to vitamin K antagonists, whose use is hampered by several limitations. These new drugs will potentially increase the number of patients treated according to guidelines, thus preventing a remarkable proportion of strokes.

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## INTRODUCTION

Stroke is a leading cause of death and disability worldwide[1] and its burden on global health-care expenditure is supposed to increase, mainly because of the ageing population. Incidence of stroke is higher than that of myocardial infarction in some regions[2] and in spite of a mild reduction of incidence in developed countries in the last four decades, a rise of cerebrovascular events has been observed in low-income countries[3]. Stroke has also substantial indirect costs related to complications, such as post-stroke dementia, depression, falls, fractures and epilepsy.

Almost 90% of all cerebrovascular events depends on few medical conditions, including bad habits (*e.g.*, smoking, physical inactivity, diet, alcohol excess, stress) as well as arterial hypertension, cardiac diseases, diabetes, and high ratio of apolipoprotein B to apolipoprotein A1[4]. Lifestyle modifications as well as medical and surgical preventive strategies may, therefore, reduce the risk of stroke. Although primary prevention remains a milestone, effective secondary prevention plays also a fundamental role in this regard. About 30% of strokes occur in individuals with a previous transient ischaemic attack (TIA) or stroke[2], and more than 50% occur in subjects with previous vascular events of any kind[5]. Much progress has been made in the last decades in stroke prevention, and there is now evidence from randomized trials that several treatments are effective in preventing recurrences (Figure 1). In particular, at least 80% of recurrent events might be prevented with the use of a comprehensive approach that includes dietary modifications, physical exercise, blood-pressure lowering drugs, antiplatelet therapy, and statins[6].

The effect of all these different interventions on stroke risk can only be derived from population-based studies. In this regard, it has been shown that the risk of recurrent stroke in all patients in a United Kingdom population-based study after a first-ever TIA or non-disabling stroke in the period 1981-1986 was higher than the risk of the same population during 2002-2010[5]. These findings were corroborated by a recent review[7] of randomized controlled trials in secondary prevention after stroke and vascular events. Recurrent stroke and vascular events rates declined substantially in the last 5 decades, mostly because of improved blood pressure (BP) control and more frequent use of antiplatelet therapy.

In this review we will focus on medical treatment for secondary prevention in patients with first-ever TIA or ischaemic stroke and separate acute secondary prevention (*i.e.,* prevention in the first hours or days) from long-term secondary prevention. We will also deal with surgical procedures and endovascular techniques for prevention of recurrences in carotid stenosis as well as therapeutic strategies in stroke associated with patent foramen ovale (PFO).

## EARLY SECONDARY PREVENTION

### Background

The risk of recurrent stroke is higher during the first 90 d after an index event, longitudinal studies showing that approximately 1 out of 2 recurrences in the first year post-stroke occurs within the first 90 d[8-12]. The 90-d risk of recurrence after a TIA has been reported to be as high as 17%, with the greatest risk in the first week[13,14]. In this regard, prospective studies have shown that stroke risk after a TIA or minor stroke is 3.1% (95%CI: 2.0-4.1) at 2 d and 5.2% (3.9-6.5) at 7 d[7,15]. After a stroke or TIA, the risk of recurrence is still high in the first month (4%) and year (12%) but falls down to about 5% per year thereafter[15]. For this reason, secondary prevention should be started urgently in the acute phase after TIA or minor stroke. Identifying patients at higher risk of recurrence is, therefore, crucial. For such a purpose several risk scores have been created. Comparison between these scores is hampered by differences in index event, methodology and factors taken into account. To date, the ABCD2 score combined with diffusion-weighted imaging (DWI) data (ABCD2I)[16-19] appears as the best predictor of the early risk of stroke after a TIA. The recently validated ABCD3I score, including carotid stenosis in addition to abnormal DWI, showed even higher predictive power, although c-statistics were less convincing in the validation set[20].

The evidence for acute treatment after TIA and minor stroke is derived from few small randomized trials, extrapolation of results from larger trials in other settings, and on two non-randomized studies of a combination of preventive treatment started urgently[21]. The Early Use of Existing Preventive Strategies for Stroke (EXPRESS) study[22] was nested in a population-based study of all acute TIAs and strokes in the Oxfordshire and compared urgent assessment and treatment (phase 1) with appointment-based clinic assessment and primary care-initiated treatment (phase 2). Results demonstrated a reduction of the 90-d risk of stroke recurrence from 10.3% in phase 1 to 2.1% in phase 2. Similarly, in the SOS-TIA study[23], urgent intensive treatment was associated with a better prognosis and a low rate of recurrent events.

Based on the results of these studies, guidelines now recommend that patients with suspected TIA should be quickly referred to a TIA clinic or to a medical centre providing rapid evaluation and appropriate treatment. In such patients, urgent vascular imaging (ultrasound, cranial tomography angiography or magnetic resonance angiography) in addiction to standardized emergency diagnostic tests, including cranial tomography or magnetic resonance imaging, electrocardiography and laboratory tests are recommended[24,25].

However, the relative contribution of antithrombotic therapy, revascularization techniques, statins and antihypertensive drugs in stroke prevention remains uncertain.

### Antithrombotic treatment

Aspirin is the only antiplatelet drug evaluated in the acute phase of stroke. Given within 48 h of onset of major ischaemic stroke it reduces 14-d morbidity and mortality, mostly by reducing the risk of early recurrent stroke[26]. However, the optimal dose of aspirin has not been stud-

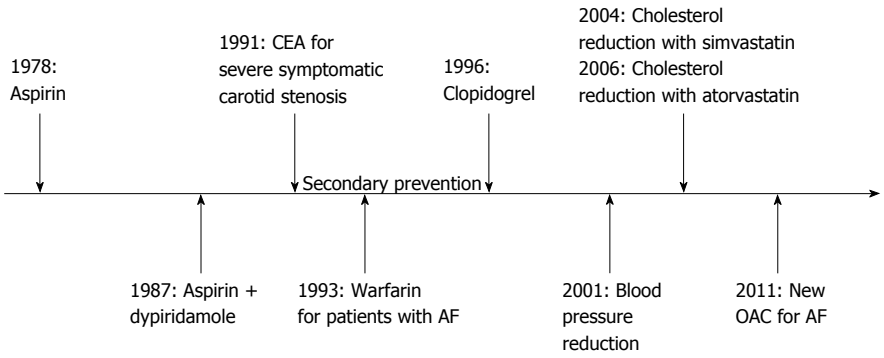


**Figure 1 Medical and surgical treatments which have been proved to be effective for secondary prevention after stroke or transient ischaemic attacks in the last decades.** OAC: Oral anticoagulants; CEA: Carotid endarterectomy; AF: Atrial fibrillation.

ied in head-to-head comparison. A recent meta-analysis by Chen *et al*[27], including studies in which daily doses of aspirin ranged between 160 to 325 mg, found no heterogeneity of effect on different outcomes. Similarly, optimal timing of initiation of aspirin therapy has not been studied in randomized trials. Though unproven, it seems reasonable to start aspirin therapy as soon as possible, ideally within 48 h of stroke onset. Furthermore, aspirin effect may be negligible in patients at high risk for early recurrence, such as those with stroke or TIA due to arterial thromboembolism[28]. Alternative approaches in these cases, based on the combination of multiple antiplatelet agents with different mechanisms of action, seem, therefore, reasonable. The combination of aspirin and clopidogrel is, actually, more effective than aspirin alone in preventing vascular recurrences and death in acute coronary syndromes[29]. In line with these findings, there is some evidence that a short course of more intensive antiplatelet treatment might be effective also in the acute phase after TIA and minor stroke[21]. Among 731 patients with acute ischaemic stroke or TIA (onset within 3 h) enrolled in five small trials, the addition of clopidogrel to aspirin was associated with a nonsignificant trend toward a reduction in recurrent stroke and an increase in major bleedings[30]. Although dual therapy seems not effective in lacunar strokes[31], it may be more effective than single antiplatelet therapy in preventing early recurrence in patients with acute symptomatic atherothrombosis, according to the recent Clopidogrel in High-risk patients with Acute Nondisabling Cerebrovascular Event trial[32] conducted on a Chinese stroke population. Because of these inconsistencies, the results of three ongoing trials investigating the safety and efficacy of dual and triple antiplatelet therapy[33-35] are required before definite recommendations can be made.

### Antihypertensive agents

An increase of BP values (even in previously normotensive patients) during the acute phase of ischaemic stroke, followed by a spontaneous decline starting within 90 min of symptoms onset[36] is common finding after stroke. Although extreme arterial hypertension is clearly detrimental, as it might exacerbate oedema and favor the haemorrhagic transformation of ischemia, a moderate increase of BP values during the acute phase of stroke may improve the perfusion of the ischaemic tissue, leading to better outcomes. Conversely, an impairment of cerebral perfusion by excessive BP lowering may exacerbate the ischaemic damage, an effect that is even more evident in conditions of chronically damaged autoregulation, such as in elderly subjects, or in cases of severe carotid stenosis[37]. Unfortunately, the ideal range of BP values to be maintained early after stroke has not yet been determined. Larger trials with well-defined criteria are needed. At present, many clinicians in their daily clinical practice consider a BP greater than 220/120 mmHg an indication to active BP lowering[36]. However, this threshold might be different in cases with minor cerebral damage, such as TIA or minor strokes, where the reduction of cerebral perfusion is likely to be of less concern. In line with this view, antihypertensive therapy started immediately after the acute event did not negatively influence the outcome in the EXPRESS[22] and SOS-TIA[23] studies.

Although antihypertensive medications are effective in the long-term secondary prevention after stroke and TIA[38], the issue of early BP lowering after a cerebrovascular event is still uncertain, recent trials suggesting no benefit[39-41]. In the Controlling Hypertension and Hypotension Immediately Post-Stroke trial[39], oral nimodipine started within 48 h after ischaemic stroke onset in 350 patients was associated with a higher mortality rate and similar functional outcome. The Scandinavian Candesartan Acute Stroke Trial[41], comparing candesartan to placebo given within 30 h of ischaemic or haemorrhagic stroke, showed no difference in the composite endpoint of vascular death, myocardial infarction or stroke after 6-mo follow-up. Similarly, in the Continue or Stop Post-Stroke Antihypertensive Collaborative Study trial[40], continuation of antihypertensive therapy during hospitalization for ischaemic stroke was not associated with a reduction of 6-mo mortality or cardiovascular event rate, when compared to discontinuation of preexisting antihypertensive drugs.

### Lipid lowering agents

In the acute phase after a cerebrovascular event statins might be beneficial because of their pleiotropic effects. These include a neuroprotective effect, limiting damage

and improving recovery (in particular for major strokes), as well as an antithrombotic effect, which in turns may prevent early recurrences (especially in TIAs and non disabling strokes). The benefits related to neuroprotective effects are perhaps more evident in the first 3 to 7 d after stroke and with higher doses of statins, as suggested by some observational studies and clinical data[42-44]. However, data from the fast assessment of stroke and transient ischaemic attack to prevent early recurrence pilot trial are discordant[45]. In this recent trial patients who presented with TIA or minor stroke within 24 h of symptoms onset were randomly assigned to simvastatin or placebo. No evidence of benefit of simvastatin was shown in the primary outcome of recurrent strokes within 90 d. Furthermore, a recent meta-analysis, including 8 randomized controlled trials, found insufficient evidence to establish safety and effectiveness (also in terms of secondary prevention) of statin use in the acute phase after ischaemic stroke[46]. Similarly, initiation of statin therapy within 14 d following the onset of acute coronary syndromes does not reduce significantly the risk of death, myocardial infarction or stroke up to 4 mo[47]. The major ongoing studies aimed at further assessing the benefit of early statin administration mainly focus on functional outcomes instead on the prevention of early recurrences[46].

### *Glycemic control*

Hyperglycemia is common during acute ischaemic stroke, being present in more than 40% of patients, mostly among those with a history of diabetes[48,49], and it is associated with worse outcome. So far, only 1 randomized efficacy trial of hyperglycemia treatment in acute stroke, the Glucose-Insulin-Stroke Trial-UK[50], has been reported. Nine hundred and thirty-three patients with acute ischaemic stroke within 24 h of symptom onset, not previously treated with insulin, were randomized to unblended intravenous treatment with insulin, potassium, and glucose versus saline for 24 h. Although the results of this trial in terms of clinical outcomes were neutral, the key questions remain unanswered because of the trial design. Thus, the definitive efficacy and safety of earlier and greater reductions in serum glucose levels during acute ischaemic stroke remain to be studied. In the meantime, it is reasonable to treat hyperglycemia to maintain blood glucose levels in a range of 140 to 180 mg/dL, preventing hypoglycemia with close monitoring, according to the current American Diabetes Association guidelines for glycemic contro[51]. No data are available for the treatment of diabetes in the setting of TIA or minor strokes, but it seems reasonable to apply the same recommendation as in major strokes. Furthermore, information concerning the value of hyperglycemic control in early secondary prevention of stroke, as well as in long-term secondary prevention, is lacking.

## LONG-TERM SECONDARY PREVENTION

Evidence from randomized controlled trials for long-term secondary prevention of ischaemic stroke is more robust than for acute treatment.

### *Antithrombotic treatment*

Ischaemic stroke is a heterogeneous clinical condition and the end-result of different underlying mechanisms: atherothrombosis (30%), cardioembolism (20%), small-vessel disease (25%). Approximately 25% of cases have no detectable cause (cryptogenic). Elucidating the mechanism of stroke is crucial to select the most appropriate therapeutic strategy and prevent further events.

**Cerebral ischaemia of arterial origin:** Antiplatelet agents are the cornerstone for secondary prevention of ischaemic events in patients with noncardioembolic strokes. Commonly used antiplatelet agents are aspirin, dipyridamole , and clopidogrel. Aspirin is an irreversible inhibitor of cyclooxygenase-1, which in turn inhibits the formation of thromboxane A2. Dipyridamole increases cyclic AMP by inhibiting platelet phosphodiesterase E5. Clopidogrel is a thienopyridine P2Y12 adenosine diphospate receptor blocker. Ticlopidine, which has the same mechanism of action as clopidogrel, is no longer recommended because of its side effects.

Aspirin, the most commonly used antiplatelet drug, is recommended for secondary prevention after cerebral ischaemia of arterial origin at a dose of 30-325 mg daily[24,25,52]. In order to reduce bleeding complications, after the acute phase (generally 1 or 2 wk after the event) the aspirin dose may be reduced to 75-100 mg[52]. However, in a meta-analysis of trials for secondary prevention of stroke, the relative reduction in stroke risk achieved by aspirin is small, being only 13%[53]. To date, the three large trials[54-56] which investigated whether vitamin K antagonists (VKAs) are more effective than aspirin for this indication, showed no difference, irrespective of the intensity of anticoagulation. Several trials have studied other antiplatelets as an alternative or in addition to aspirin. The second European Stroke Prevention Study (ESPS2) trial[57] and the European/Australasian Stroke Prevention in Reversible Ischaemia Trial (ESPRIT)[56] investigated the efficacy of the combination of aspirin plus dipyridamole versus aspirin alone. Meta-analysis showed a relative risk reduction of 18% in the vascular events in favour of the combination therapy[58]. In the Clopidogrel versus Aspirin in Patients at Risk of Ischaemic Events (CAPRIE) study, clopidogrel achieved a non-significant 7.3% reduction in relative risk of the composite outcome of stroke, myocardial infarction or vascular death, when compared to aspirin[59]. The combination of aspirin plus clopidogrel was compared with clopidogrel alone in the Aspirin and Clopidogrel compared with Clopidogrel Alone after Recent Ischaemic Stroke or TIA in High-risk Patients (MATCH) trial[60] and with aspirin monotherapy in the Clopidogrel for High Atherothrombotic Risk and Ischaemic Stabilization, Management, and Avoidance (CHARISMA) trial[61]. Both trials showed higher rates of bleeding complications in the dual antiplatelet arm which overcome any beneficial effect. A more recent study, comparing clopidogrel plus aspirin with aspirin

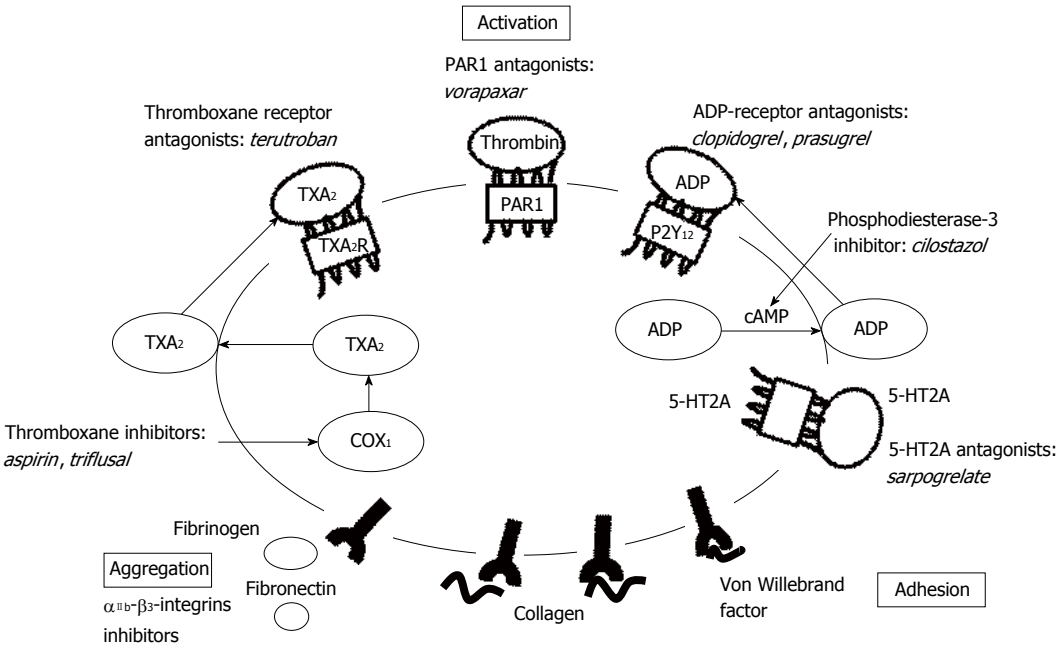


**Figure 2 Old and new antiplatelet agents and their mechanism of action.** PAR1: Proteinase activated receptor 1; ADP: Adenosine diphosphate; $TXA_2$: Thromboxane $A_2$; cAMP: Cyclic adenosine monophosphate; $COX_1$: Cyclooxygenase-1; 5-HT2A: 5-hydroxytryptamine (serotinin) receptor 2A.

alone in recent lacunar strokes confirmed these results[31]. Although in indirect comparisons the combination of aspirin plus dipyridamole was more effective than clopidogrel[62], the non-inferiority Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) trial did not confirm this result, showing a similar rate of recurrent stroke in both treatment groups[63]. Furthermore, the rates of discontinuation and of major systemic hemorrhages were higher in the aspirin plus dipyridamole group.

Current therapies have several limitations, including a weak inhibition of platelet function with modest clinical effect, blockade of only one platelet pathway, slow onset of action (*i.e.,* for clopidogrel), high bleeding risk, and interpatient response variability with poor inhibition of platelet activity. The concept of "antiplatelet resistance" has been proposed for those patients experiencing recurrences during correct antiplatelet therapy. It occurs when the drug biochemically fails to inhibit platelet activation, as measured by *in vitro* platelet function assays. Proposed mechanisms responsible for this phenomenon include drug-drug resistance and genetic polymorphisms of antiplatelet metabolism or drug-receptor site. However, because of the lack of a standardized assay and the poor correlation between antiplatelet resistance and recurrent clinical events, currently there is no indication to screen patients for antiplatelet resistance[64,65]. For clopidogrel, in particular, it seems prudent to avoid interactions of drugs with a well-known effect on hepatic cytochrome p450, as it has been demonstrated that these drugs might affect clopidogrel metabolism[64]. Consequently, in the last years, other antiplatelet agents with different mechanisms of action have been investigated and compared to aspirin (Figure 2). Triflusal is a thromboxane inhibitor structurally related to aspirin with a similar efficacy in preventing vascular events, but a lower risk of bleeding, in spite of more non-haemorrhagic adverse gastrointestinal events[66]. Cilostazol is a phosphodiesterase-3 inhibitor which represents a promising alternative to aspirin in Asian people with stroke. A meta-analysis including two randomized clinical trials concluded that cilostazol is more effective than aspirin in the prevention of vascular events secondary to stroke, causes more frequently minor adverse effects, but less frequently bleeding complications[67]. The Prevention of Cardiovascular Events in Ischaemic Stroke Patients with High Risk of Cerebral Haemorrhage (PICASSO) trial is an ongoing study aimed at randomizing patients with previous stroke and high bleeding risk (*i.e.*, patients with a symptomatic or asymptomatic old cerebral haemorrhage) to aspirin or cilostazol (and to probucol)[68]. Sarpogrelate, a selective inhibitor of 5-hydroxytryptamine receptors, has been investigated in comparison with aspirin and turned out to be non-inferior to the latter for prevention of stroke recurrences, with fewer bleeding events[69]. Terutroban, which is a specific antagonist of the thromboxane A2 receptor, was no more effective than aspirin for prevention of stroke in the large Prevention of Cerebrovascular and Cardiovascular Events of Ischaemic Origin with Terutroban in Patients with a History of Ischaemic Stroke or TIA (PERFORM) trial[70]. Finally, vorapaxar is a novel antiplatelet agent that selectively inhibits the cellular action of the thrombin through antagonism of proteinase activated receptor 1. In a recent trial[71] in which vorapaxar was compared to placebo in patients with vascular events, it reduced the risk of cardiovascular death or ischaemic events in patients with stable atherosclerosis who were receiving standard therapy. However, it increased major bleedings, including intracranial haemorrhages.

On the basis of these trials, international guidelines

**Table 1 Antiplatelet therapy after non-cardioembolic stroke or transient ischaemic attack**

| | Options | Recommendation |
|---|---|---|
| AHA/ASA[25] | Aspirin (50-325 mg); aspirin plus dipyridamole; clopidogrel | Aspirin, aspirin plus dipyridamole or clopidogrel |
| ESO[24] | Aspirin; aspirin plus dipyridamole; clopidogrel; triflusal | Clopidogrel or aspirin plus dipyridamole |
| ACCP[52] | Aspirin (75-100 mg); clopidogrel; aspirin plus dipyridamole; cilostazol | Clopidogrel or aspirin plus dipyridamole |

AHA/ASA: American Heart Association/American Stroke Association; ESO: European Stroke Organisation; ACCP: American College of Chest Physicians.

**Table 2 $CHADS_2$, $CHA_2DS_2VASC$, and HAS-BLED risk stratification methods**

| | |
|---|---|
| $CHADS_2$ | |
| Congestive heart failure | 1 |
| Hypertension | 1 |
| Age ⩾ 75 yr | 1 |
| Diabetes mellitus | 1 |
| Stroke, TIA, or thromboembolism | 2 |
| Maximum score | 6 |
| $CHA_2DS_2VASc$ | |
| Congestive heart failure/LV dysfunction | 1 |
| Hypertension | 1 |
| Age ⩾ 75 yr | 2 |
| Diabetes mellitus | 1 |
| Stroke, TIA, or thromboembolism | 2 |
| Vascular disease (previous MI, PAD, or aortic plaque) | 1 |
| Age 65-74 yr | 1 |
| Sex category (female sex) | 1 |
| Maximum score | 9 |
| HAS-BLED | |
| Hypertension | 1 |
| Abnormal renal/liver function | 1 point each |
| Stroke | 1 |
| Bleeding history or predisposition | 1 |
| Labile INR | 1 |
| Elderly (age > 65 yr) | 1 |
| Drugs (*e.g.*, concomitant antiplatelet/NSAID) or alcohol | 1 point each |
| Maximum score | 9 |

TIA: Transient ischaemic attack; LV: Left ventricular; MI: Myocardial infarction; PAD: Peripheral artery disease; NSAID: Nonsteroidal inflammatory drugs; INR: International normalized ratio.

still vary, but most recommend aspirin plus dipyridamole or clopidogrel as first line therapy in long-term secondary prevention of stroke. If these are not available or contraindicated, aspirin monotherapy, triflusal and cilostazol are alternatives (Table 1). However, even if both aspirin plus dipyridamole and clopidogrel alone are statistically superior compared to aspirin alone, clinical superiority is at best minimal. Furthermore, if tolerance profile is included in judgment of efficiency, then aspirin or clopidogrel monotherapy should be advised as the first-line therapy, since aspirin plus dipyridamole and aspirin plus clopidogrel carry higher rates of discontinuation and of bleeding complications, respectively. If also economic considerations are taken into account, then aspirin therapy remains the best choice[72].

Patients who present with a first or recurrent stroke are commonly already on antiplatelet therapy. To date, for patients experiencing a stroke during aspirin therapy, there is no evidence that increasing the dose of aspirin provides additional benefit. Moreover, although alternative antiplatelet agents are often considered, no single agent or combination has been studied. In such a situation it is important to take into account the compliance to therapy, as well as other vascular risk factors and possible different stroke mechanisms.

**Cerebral ischaemia caused by atrial fibrillation:** Roughly 20% of all TIAs and ischaemic strokes have a cardiac origin, most commonly caused by atrial fibrillation (AF). AF is associated with a five-fold increased risk of stroke, the yearly risk varying from 0.2% in patients with AF alone to more than 10% in individuals with other risk factors[73]. The major risk factors for stroke in AF patients are a history of stroke or TIA, advancing age, hypertension, systolic BP (SBP) > 160 mmHg, and diabetes. These and other risk factors have been used to create several scores for stroke risk calculation, in order to recognize patients who could benefit from anticoagulation. The most widely used model for stratification of stroke risk is $CHADS_2$ score (Table 2). However, although the score is simple and has been externally validated it does not reliably discriminate between patients with low risk, who do not need anticoagulation, and patients at intermediate risk, who do[73]. This has prompted to the development and the external validation in independent cohorts of a new score, the $CHA_2DS_2VASc$ score (Table 2). Because of the inclusion of newly recognized risk factors for stroke, this score allows a better stroke risk stratification in patients who are considered at low or intermediate risk for stroke[74]. Beside quantification of stroke risk, another important issue is the stratification of bleeding risk associated with anticoagulation. Among the schemes created for such a stratification, the most frequently used is the HAS-BLED score (Table 2), which was originally derived from a cohort of 3456 patients with AF and a one-year follow-up[75]. HAS-BLED score, which has been validated in external cohorts, correlates with the risk of intracranial bleeding, a score of more than 3 identifying patients at high bleeding risk. In these conditions, it does not necessarily mean that anticoagulation should not be initiated but that such therapy warrants special caution, control of modifiable risk factors, and close monitoring[73]. Difficulty arises in clinical practice when patients have key risk factors for both ischaemic stroke and major bleeding (*e.g.*, age, hypertension, or previous stroke). Among these factors, previous stroke and increasing age are more strongly associated with ischaemic stroke than with intracranial bleeding[76,77].

Recommendations for patients with AF and a his-

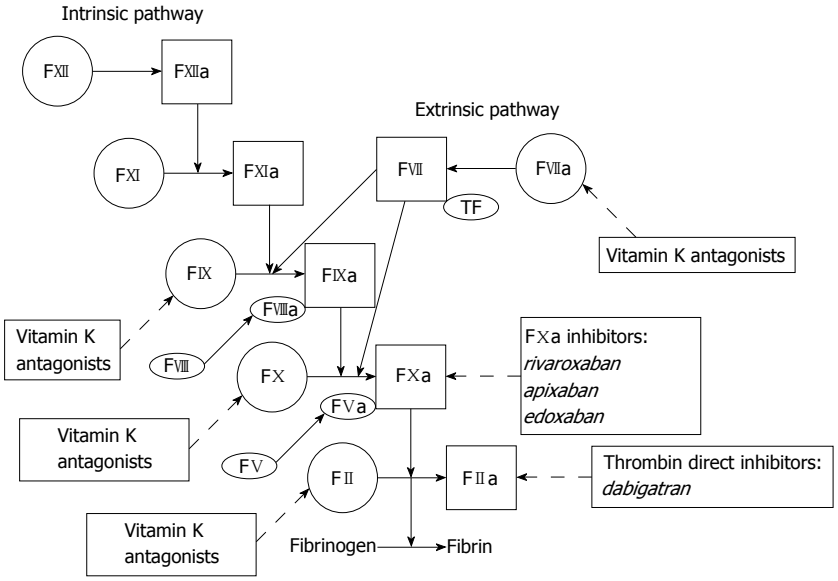


**Figure 3 Vitamin K antagonist and new anticoagulant mechanism of action.** F: Factor.

tory of stroke and TIA are based on the pooled effect of anticoagulation in primary and secondary prevention studies, as data on secondary prevention are limited to few studies. The first of these studies is the European AF Trial[78], in which patients were randomly allocated to VKAs, 300 mg/d-aspirin, or placebo. VKAs turned out to be the most effective treatment, despite a higher number of major haemorrhages. The findings were confirmed in patients with previous TIA or ischaemic stroke enrolled in the Stroke Prevention in AF Ⅲ trial[79], which was halted early because patients assigned to fixed low-dose VKA (INR 1.2-1.5) plus aspirin 325 mg daily had four times more ischaemic events, compared to those allocated to regular VKA dose. A further trial in patients with recent cerebral ischaemia and AF found 15% reduction of recurrences with VKA than with indobufen[80]. Adjusted-dose warfarin (target INR 2.0-3.0) turned out to be significantly more effective than the combination of aspirin plus clopidogrel in the AF Clopidogrel Trials with Irbesartan for prevention of Vascular Events (ACTIVE)[81,82]. ACTIVE W was stopped prematurely because of an excess of primary outcomes in the aspirin plus clopidogrel group than in warfarin group. ACTIVE A included patients who were not eligible for VKAs and showed a small advantage for the combination of clopidogrel and aspirin compared to aspirin alone in preventing ischaemic events, but with more bleedings.

Despite their effectiveness, VKAs have many limitations, including slow onset and offset of action, interindividual variability in their effect, narrow therapeutic index, food and drug interactions and reduced synthesis of all vitamin K-dependent proteins[83]. It has been calculated that at least a third of patients with AF who are at risk for stroke are either not started on VKAs therapy or discontinue the therapy. These limitations have prompted to the development and assessment of other agents that target the coagulation cascade at sites different from those of classic VKAs: factors Xa and direct thrombin inhibitors (Figure 3). The first of these agents, Ximelagatran, a factor Xa inhibitor, was investigated in two trials[84,85]. It resulted as effective as warfarin in prevention of stroke with fewer bleedings, but it turned out to be hepatotoxic and was then withdrew. Dabigatran, a thrombin inhibitor, was compared to dose-adjusted warfarin in the Randomized Evaluation of Long-term Anticoagulation Therapy (RE-LY) trial[86] in doses of 110 and 150 mg twice daily in approximately 12000 patients, 20% of whom had a history of TIA or stroke. The lower dose of dabigatran was non-inferior to warfarin in reducing the rate of stroke or systemic embolism, while the higher dose was superior. Both doses significantly reduced major bleedings compared with warfarin in patients younger than 75 years, whereas in elderly patients the lower dose was associated with a similar rate and the higher dose with an increased rate of major bleedings[87]. The factor Xa inhibitor rivaroxaban (20 mg daily) was compared with warfarin in the Rivaroxaban-Once daily, oral, direct factor Xa inhibition Compared with vitamin K antagonism for prevention of stroke and Embolism Trial in AF (ROCKET-AF)[88]. The study population in this trial was at high risk of stroke: 55% of patients had previous stroke or TIA and 90% had either a previous stroke or TIA, or three or more risk factors for stroke (mean $CHADS_2$ score 3.5). Rivaroxaban was non-inferior to warfarin for the prevention of stroke or systemic embolism and had a similar adverse effect profile. In the Apixaban for Reduction in Stroke and Other Thromboembolic Events in AF trial[89] apixaban was better than warfarin in reducing the rate of embolic events and was associated with significantly less major bleedings, less intracranial haemorrhages and lower mortality. Finally, the Apixaban *vs* Aspirin to Prevent Stroke (AVERROES) trial investigated apixaban (5 mg twice daily) versus aspirin (81-324 mg daily) in patients with AF who were unsuitable or unwilling to receive VKAs[90].

**Table 3 Anticoagulation therapy after stroke or transient ischaemic attack in patients with atrial fibrillation**

| | Recommendation | Alternatives in patients unsuitable to anticoagulation |
|---|---|---|
| AHA/ASA[25] | Warfarin (INR 2.0-3.0) | Aspirin |
| ESO[24] | Warfarin (INR 2.0-3.0) | Aspirin plus dypiridamole |
| ACCP[52] | Dabigatran (150 mg twice daily) | Aspirin plus clopidogrel |

AHA/ASA: American Heart Association/American Stroke Association; ESO: European Stroke Organisation; ACCP: American College of Chest Physicians; INR: International normalized ratio.

The rate of stroke or systemic embolism was significantly lower with apixaban than with aspirin, whereas the rates of major bleeding, intracranial haemorrhage, gastrointestinal bleeding, and myocardial infarction were similar in both groups. Another large phase Ⅲ trial with a new anticoagulant, edoxaban, is ongoing[91]. The proportion of patients with AF who were enrolled in all these trials and had a previous stroke or TIA varied between 14% in AVERROES and 55% in ROCKET-AF. In each trial, the absolute rate of stroke or systemic embolism was higher in patients with a previous stroke or TIA than in those without, but the relative effect of each new anticoagulant compared to warfarin in the prevention of stroke or systemic embolism was consistent among patients with or without previous cerebral ischaemic events. Similarly, the relative effect of each new agent on major bleeding did not differ[92-95]. Indirect comparisons suggest that the relative effects of each of the new oral anticoagulants compared with warfarin were consistent for stroke and systemic embolism, haemorrhagic stroke, and mortality, but not for myocardial infarction and extracranial major bleeding[96-102]. Similar results have been confirmed in a recent meta-analysis of data from 12 phase Ⅱ and phase Ⅲ randomized controlled trials comparing new oral anticoagulants with warfarin[103].

The majority of current guidelines still regard VKAs at INR 2.0-3.0 to be the standard treatment after cerebral ischaemia of cardiac origin, including AF, for patients who are suitable for such therapy, with few exceptions, on the basis of recent findings concerning new anticoagulants[24,25,52] (Table 3). Although these drugs have some advantages compared to warfarin, the unknown long-term safety profile, the absence of an antidote in the case of bleeding and their costs are important issues to consider.

### *Antihypertensive agents*

Long term management of hypertension significantly reduces the risk of recurrent events. A meta-analysis of 7 randomized controlled trials including 15527 patients with TIA or stroke randomly allocated to treatment group within 1-14 mo after the event, showed reduction of recurrent stroke, myocardial infarction and vascular death[38]. Benefit was present irrespective of a previous diagnosis of hypertension and risk reduction was greater with larger BP lowering. However, there is uncertainty regarding the class of drugs to prefer because of the small number of trials included. Significant reductions in recurrent stroke were seen with diuretics alone and in combination with angiotensin-converting enzyme inhibitors (ACEIs) but not with β-blockers (BBs) or ACEIs used alone; nonetheless, statistical power was limited, particularly for the assessment of β-blockers, and calcium channel blockers (CCBs) and angiotensin receptor blockers (ARB) were not evaluated in any of the included trials. One of the largest trial included in the meta-analysis, the PROGRESS trial, showed a 26% reduction of the risk of stroke with the combination of perindopril and indapamide compared to placebo[104]. Patients were included regardless of baseline BP and benefits were seen even in those patients who were normotensive before admission. Since this meta-analysis, 2 additional large-scale randomized trials were published: the Morbidity and Mortality after Stroke, Eprosartan Compared to Nitrendipine for Secondary Prevention (MOSES) trial[105], and the PRoFESS trial[106]. In the MOSES, patients with an ischaemic event in the previous 2 years were randomly allocated to eprosartan (an ARB) or nitrendipine (a CCB). The risk of stroke was lower in the former group, but the benefit was only due to fewer TIAs, with no significant effect on stroke[105]. The PRoFESS failed to show a protective role of telmisartan, an ARB, in stroke prevention. Similarly, another study found no difference in a composite outcome of death from vascular causes, myocardial infarction, stroke or hospitalization for heart failure between patients with previous vascular event of any kind or diabetes randomly allocated to telmisartan or ramipril[107]. The Telmisartan to Prevent Recurrent Stroke and Cardiovascular Events (TRANSCEND) trial[108] showed only a modest effect on prevention of vascular events in patients treated with telmisartan, thus confirming the lack of evidence of ARB effectiveness in stroke prevention.

On the basis of the available evidence, current guidelines recommend antihypertensive treatment in most patients with recent TIA or stroke, beyond the first 24 h, irrespective of a history of hypertension before admission, with some favouring the combination of an ACEI and a diuretic agent, on the basis of PROGRESS trial[25]. An absolute BP target and reduction are uncertain and should be individualized, but benefit has been associated with an average reduction of roughly 10/5 mmHg, and normal BP levels have been defined as > 120/80 mmHg[109].

However, recent studies have shown that visit-to-visit variability in BP and episodic hypertension are powerful risk factors for stroke, independently of mean BP, and that the benefits of some BP-lowering drugs are attributable partly to reduce variability in BP levels[110,111]. Although reductions in mean SBP are similar with different drug classes, CCBs and diuretics also reduce variability

in SBP, while BBs increase variability[112,113]. In particular, it has been shown that higher doses of CCBs reduce BP variability more than lower doses, and higher doses of BBs increase BP variability[114]. This means that combinations containing a low dose of BBs will have less adverse effects on variability in SBP and the associated risk of stroke, but low doses of CCBs will be less effective in reducing BP variability. Drug class effects on variability persist even in combination therapy.

Another issue to consider is the heterogeneity of stroke patients. Elderly patients seem to benefit most from a combination therapy of ACEIs and diuretics[115], as well as patients with renal impairment or diabetic nephropathy, in whom ACEIs and ARBs are recommended. The detrimental effect of such drugs on BP variability could be offset by the combination use with CCBs, as suggested in the Avoiding Cardiovascular Events through Combination Therapy in Patients Living with Systolic Hypertension study[116], in whom patients with hypertension and previous vascular disease, including stroke, were randomly allocated to a combination of benazepril plus amlodipine or a combination of benazepril plus hydrochlorothiazide. The former group of patients had fewer combined cardiovascular deaths and chronic kidney disease events compared to the latter.

However, direct comparisons of drugs for secondary prevention of stroke are needed in order to recommend the most appropriate BP-lowering drug regimen. More research is required to identify ideal combinations and doses of drugs for the prevention of stroke in specific patient groups with particular reference to their effect on BP variability.

### *Lipid lowering agents*

Although the association between serum lipid concentrations and ischaemic risk is weaker for stroke than for myocardial infarction[117], lipid levels modification is important in both primary and secondary prevention of stroke[118]. A recent observational study[44] showed a better functional outcome in stroke patients who received statin therapy before admission to hospital and who continued such a treatment during hospitalization. The prognosis was even better with higher doses of statins and when therapy was started earlier. Regarding secondary prevention, in a retrospective analysis of the Heart Protection Study (HPS) [119] restricted to subjects with a history of stroke, simvastatin did not significantly reduce the risk of stroke. In contrast, the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial[120] showed a 16% reduction in stroke recurrence in patients with previous stroke or TIA, not of cardiac origin, randomized to atorvastatin 80 mg daily versus placebo, within 6 mo from index event. One concern in the SPARCL trial and in the subset of patients in HPS[119] who had a stroke before randomization was a significant increase in the risk of haemorrhagic stroke in patients allocated to statin treatment[121]. Further analysis showed that much of excess risk of haemorrhagic stroke on atorvastatin therapy was in patients with a small vessel disease stroke as qualifying event, either of haemorrhagic or ischaemic type[122]. Although in the former group there was no overall benefit, in the latter the net benefit was still in favour of treatment because of a reduction in ischaemic recurrences[122]. This suggests that statin therapy should be initiate only in patients with atherosclerotic stroke or in presence of other noncerebrovascular indications, such as coronary heart disease or diabetes[123].

Another important clinical issue relates to low-density lipoprotein (LDL) concentration. In a meta-analysis of all statin trials that looked at intense LDL lowering versus standard treatment, achievement of an LDL concentration of 100 mg/dL resulted in 16% relative reduction in risk of stroke. Subanalysis of the SPARCL trial showed a 28% reduction in stroke risk with a LDL concentration lower than 70 mg/dL in comparison with a level of 100 mg/dL[124,125]. The Treat Stroke to Target trial[126] is an ongoing study aimed at unravelling the issue regarding the optimal LDL target to reach. The working hypothesis of this trial is that the achievement of an LDL concentration of 70 mg/dL is better than 100 mg/dL in patients with previous atherosclerotic TIA or stroke. The Optimal BP and Cholesterol Targets for Preventing Recurrent Stroke in Hypertensive (ESH-CHL-SHOT) trial[127] will be started soon to address this issue. Its purpose, indeed, is to investigate both SBP and LDL cholesterol levels to achieve to optimize secondary prevention. Regarding lipid-lowering treatment in particular, the patients will be randomly allocated to one of two different LDL targets, 70 to 110 mg/dL or < 70 mg/dL, using different statins, including simvastatin, pravastatin, fluvastatin, atorvastatin or rosuvastatin.

Furthermore, there is still uncertainty regarding whether to start with high-dose or low-dose statin therapy, considering benefit and potential side effects. The SPARCL trial[120] reliably showed that high-dose atorvastain at 80 mg/d reduces the risk of stroke and other cardiovascular recurrences in patients with non cardioembolic stroke. It is however unclear, in the absence of randomized comparisons in patients with stroke, if lower doses of atorvastatin are equally or less effective than the high-dose regimen. There is evidence that atorvastatin at 80 mg/d provides significant reductions in the risk of cardiovascular events and stroke beyond that provided by a 10-mg dose in patients with coronary disease[128]. However, the high-dose regimen was complicated more frequently by higher rates of adverse events and discontinuation, both in Treating to New Targets (TNT) trial[128] and SPARCL trial[120]. Moreover, the Food and Drug Administration issued a warning regarding increased risk of myopathy in patients taking simvastatin at 80 mg/d, especially if taken in combination with CCBs, which are frequently used in patients with stroke. Although the SPARCL results support the use of high-dose atorvastatin for secondary prevention of stroke, it is unclear whether the highest currently approved doses of statins have neuroprotective effects. It is also unknown if other hydroxymethylglutaryl-coenzymeA reductase inhibitors, in different doses, could be potentially effective in pre-

vention of stroke recurrences. In this regard, two trials are ongoing to investigate the combined effect of this class of drugs[127] and that of pravastatin[129] in particular in long-term secondary prevention after stroke.

On the basis of available data the American Heart Association/American Stroke Association (AHA/ASA) guidelines recommend statin therapy with intensive lipid-lowering effect for secondary prevention among patients with ischemic stroke or TIA who have evidence of atherosclerosis, an LDL-level ≥ 100 mg/dL, and who are without known coronary heart disease. They suggest, as a target, a reduction of at least 50% in LDL or, alternatively, an LDL level of < 70 mg/dL[25].

Other medications used to treat dyslipidemia include niacin, fibrates, and cholesterol absorption inhibitors. These agents can be used by patients intolerant to statins, but the evidence of their efficacy for prevention of stroke recurrence is scarce. Niacin has been associated with a reduction in cerebrovascular events[130], whereas gemfibrozil reduced the rate of total strokes among men with coronary artery disease and low levels of high-density lipoprotein cholesterol (≤ 40 mg/dL) in the Veterans Affairs HDL Intervention Trial. The latter result was no more significant when adjudicated events alone were analyzed[131].

### *Surgical procedures*

**Percutaneous PFO closure:** PFO is a common embryonic defect of the interatrial septum, being present in up to 25% of healthy people. In a meta-analysis PFO was found to be significantly associated with increased risk of stroke in younger patients with cryptogenic stroke, especially when atrial septal aneurysm (ASA) is also present[132]. This leads to the assumption that PFO may be the cause somehow associated to stroke occurrence, although it may also be an incidental finding[133]. The role of PFO in determining the risk of recurrence is uncertain, as well. In a substudy of the PFO in Cryptogenic Stroke Study (PICSS)[134] there were no differences in the rates of recurrent strokes in those with or without PFO, as well as no demonstrated effect on outcomes based on PFO size or presence of ASA. In contrast, the study of Mas *et al*[135] reported higher recurrence rates of stroke associated with both PFO and ASA, indicating that the presence of both these cardiac abnormalities was a predictor of an increased risk of recurrent stroke, whereas isolated PFO, whether small or large, was not. Thus, the natural history after cerebrovascular events in patients with PFO remains insufficiently defined. As a consequence, the optimal management strategy for treating patients with cryptogenic stroke who are discovered to have a PFO remains to be determined. Observational studies on medical treatment in patients with PFO with either antiplatelets or warfarin reported a risk of recurrent stroke or TIA ranging from 3% to 12% during the first year[136]. Both larger PFO size and a greater degree of right-to-left shunt increased the risk of paradoxical embolism in one study[136]. Of note, observational data for the comparative effectiveness of anticoagulants versus antiplatelet agents for secondary prevention after stroke associated to PFO show a significant benefit of warfarin, whereas the only relevant randomized study, coming from a subgroup of PICSS[134], found a non-significant trend in favor of warfarin over aspirin. On the basis of this evidence, AHA guidelines state that there are insufficient data to establish the superiority of warfarin over aspirin for secondary stroke prevention after stroke or TIA in patients with PFO[25]. However, warfarin might be more beneficial in presence of deep venous thrombosis or ASA, according to the European Stroke Organization guidelines[24]. Another therapeutic possibility is percutaneous PFO closure, which has supplanted surgical procedures. Case series and nonrandomized comparisons have long suggested that closure is a highly efficacious procedure and have led to the rapid off-label adoption of this intervention[137]. In contrast, recent randomized controlled trials failed to identify any statistically significant difference between closure and medical treatment for secondary prevention after stroke[138-140]. However, such trials have several limitations and low statistical power. In the Evaluation of the STARFlex Septal Closure System in Patients with a Stroke and/or TIA due to Presumed Paradoxical Embolism through a PFO (CLOSURE) trial[138] the main limitation was the low outcome rate, compared to previous observational studies, thus pointing to a failure to select patients with PFO-related events. This may, in part, be attributable to a reluctance to randomize those patients with cryptogenic stroke who appear most likely to have had a PFO-related event (*i.e.*, patients with clinical indicators of paradoxical embolism, such as large shunt, associated ASA, Valsalva maneuver at onset of stroke, or absence of any conventional stroke risk factors). Other limitations of this trial include idiosyncratic effects, such as *in situ* thrombosis or other mechanical complications, with the use of the obsolete STARFlex device, which may have increased the outcome rates in the intervention arm, as well as the short follow-up, that may be inadequate to capture the benefit of the intervention[137]. The Randomized Evaluation of Recurrent Stroke Comparing PFO Closure to Established Current Standard of Care Treatment (RESPECT) trial[139] confirmed the results of the CLOSURE trial in intention-to-treat analysis, in spite of important differences between the two studies with respect to the study design, the population included, and the device tested. In the RESPECT trial[139] the enrollment criteria were more stringent and the follow-up period was longer than in the CLOSURE. Moreover, the Amplatzer PFO Occluder, as compared to STARFlex used in the CLOSURE, was associated with higher effective closure rate. Finally, although different in terms of population and endpoints, the Percutaneous Closure of PFO in Cryptogenic Stroke (PC) trial[140] corroborated previous results. However, unlike the results of the CLOSURE trial, the results of the RESPECT and the PC trials have encouraged the supporters of PFO closure. The advocates of closure focus on the modest statistical power of these trials, the substantial relative effect size of the point estimates in both trials, the signifi-

cance of the per-protocol and as-treated analyses in the RESPECT, and arbitrariness of the conventional *P*-value threshold of 0.05. Although the controversy over efficacy may not be settled, it is worth noting that the safety profile of Amplatzer device appeared to be superior to that of STARFLex device tested in the CLOSURE. This supports the hypothesis that PFO closure, in certain conditions, could be superior to medical treatment and reinforces the need to carry on trials with more selected populations. Indeed, other trials are ongoing, such as the PFO Closure or Anticoagulants Versus Antiplatelet Therapy to Prevent Stroke Recurrence (CLOSE) trial[141] and the GORE® HELEX® Septal Occluder/GORE® Septal Occluder for PFO Closure in Stroke Patients (REDUCE) trial[142]. However, like previous analyses, these new studies are hampered by a slow and skewed enrollment since most patients assumed to be at high risk undergo percutaneous closure. Beside, stroke pathophysiology is multifactorial, and the diagnosis of PFO-mediated paradoxical embolism is presumptive. Both a PFO and a cryptogenic stroke may coexist without causal relation in a given patient. In this case, obviously, PFO closure will not reduce the risk of recurrence. Current guidelines, which are antecedent to these recent trials, do not recommend closure because of the paucity of data[25]. However, pending other trials and based on RESPECT, it would be reasonable to discuss closure with Amplatzer device in young patients (< 50 years) with a substantial shunt and a cortical infarct, in the absence of known vascular risk factors[143].

### *Carotid endarterectomy and stenting for large-artery atherosclerosis*

Beside medical therapy, the mainstays of treatment options for symptomatic large-artery atherosclerosis are carotid endarterectomy (CEA) and carotid angioplasty and stenting (CAS), the latter being emerged more recently as an alternative to surgical intervention to prevent recurrent strokes.

CEA plus medical therapy is more effective and safe than medical therapy alone in secondary prevention after stroke for symptomatic patients with > 70% carotid stenosis according to 3 major trials, the European Carotid Surgery Trial[144], the North American Symptomatic CEA Trial[145], and the Veterans Affairs Cooperative Study[146]. However, in all these trials medical therapy did not include aggressive atherosclerotic medical management based on statins, antihypertensive agents, antiplatelets other than aspirin, and smoking cessation, thus meaning that the benefit of surgery today may be less than in the early trials. Uncertainty exists for patients with symptomatic stenosis in the range of 50% to 69%, for whom CEA should be considered only with appropriate case selection when the risk-benefit ratio is favorable for the patient. The timing of CEA after an acute event is controversial, with experts advocating waiting anywhere from 2 to 6 wk[25]. However, early surgery (< 2 wk) turned out to be beneficial, according to pooled analysis of trials. Furthermore, early intervention (< 3 wk) may be beneficial and safe in low-risk patients with TIA and minor strokes, and in those without haemorrhagic transformation[25].

CAS is a percutaneous, less invasive procedure that has emerged as an interesting alternative to CEA for the treatment of extracranial carotid artery disease, especially thanks to the advances in endovascular technology, including embolic protection devices (EPD) and improved stent design. Complication rates are comparable to CEA. Although advantages related to the less invasive procedure, CAS durability remains unproven. The largest randomized trial in symptomatic patients, the Carotid and Vertebral Artery Transluminal Angioplasty Study 2 trial[147], showed comparable outcome between the two procedures, also in the long-term follow-up. The Stent-Supported Percutaneous Angioplasty of the Carotid Artery Versus Endarterectomy trial[148] which was ended after the second interim analysis owing to the low rate of recruitment, showed similar rates of periprocedural stroke or death and ipsilateral ischaemic stroke up to 2 years between CEA and CAS. However, the fact that only 27% of patients with CAS in this study used EPD may have skewed the outcome. Because EPD reduce periprocedural stroke rates, they have been required in all stenting trials since then. The Stenting and Angioplasty with Protection in Patients at High Risk for Endarterectomy trial[149] randomized both symptomatic and asymptomatic high risk patients, showing that CAS was not inferior to CEA. However, a high percentage of patients were asymptomatic (70%), thus limiting the applicability of the results to symptomatic ones. The Endarterectomy Versus Angioplasty in Patients with Symptomatic Severe Carotid Stenosis trial[150] showed higher rates of periprocedural stroke or death at 30 d and within 4 years, as well. However, the results of this trial are influenced by the limited experience of the operators, the use of five different stents and seven different cerebral protection devices, and the fact that EPD were optional in the early phase of the study. The most important trial on this topic is the Carotid Revascularization Endarterectomy Versus Stenting Trial[151], which included patients with both symptomatic and asymptomatic carotid disease in similar proportions. It demonstrated that both procedures had similar safety and efficacy profiles for all patients, according to gender and the presence or not of previous cerebrovascular events. However, patients aged 70 years or older had better outcomes with CEA for the primary endpoint (any stroke, death or myocardial infarction (MI) within 30 d and ipsilateral stroke during long-term follow-up) and less adverse events. Younger patients had greater benefit from CAS than older subjects. The CAS group had a greater percentage of patients with stroke within the first 30 d after the procedure, but a lower rate of MI events. In trials of symptomatic patients, stenting was associated with a significantly higher risk of 30-d stroke or death, particularly in people over 70 years, compared to CEA, whereas the long-term rate of ipsilateral stroke were similar for both interventions[152]. However, stenting was associated with less MI and cranial nerve palsies.

On the basis of these studies, current AHA/ASA

guidelines recommend that patients who have experienced a TIA or stroke within the past 6 mo and have ipsilateral carotid stenosis of 70%-90% would be candidates for CEA if the perioperative morbidity and mortality risk is less than 6%. In symptomatic patients with a 50%-60% stenosis, the decision to perform CEA depends on factors such as age, comorbidities, and sex. If a patient is a good candidate for CEA, intervention within 2 wk is recommended[25]. To date, CAS is considered an alternative option for symptomatic patients with carotid stenosis of more than 70% as determined by noninvasive imaging or more than 50% as determined by catheter angiography. CAS is also an alternative in patients deemed at high risk for surgical intervention. High risk is defined by presence of severe comorbidites and challenging anatomical features (such as prior neck intervention or irradiation, postendarterectomy restenosis, surgical inaccessible lesion, contralateral carotid occlusion, contralateral vocal cord palsy, or the presence of tracheostomy)[25]. Patients are also recommended to optimize medical therapy with antiplatelets, statins and risk factor modifications.

### *Angioplasty and stenting for intracranial atherosclerosis*

According to the Stenting versus Aggressive Medical Therapy for Intracranial Arterial Stenosis (SAMMPRIS) trial[153], angioplasty and stenting of intracranial stenosis did not reduce the risk of recurrent stroke compared with aggressive medical therapy, including dual antiplatelets, statins and antihypertensive agents.

## CONCLUSION

Secondary prevention with antiplatelet and antihypertensives medications, statins and anticoagulants as appropriate, should be initiated urgently after TIA or minor strokes because of the high risk of early stroke recurrence. For long-term prevention, aspirin plus dipyridamole or clopidogrel must be proposed as first-line approach after cerebral ischaemia of arterial origin. For cardioembolic strokes in patients with non-valvular AF new anticoagulation agents are challenging the current standards of VKAs. Lipid and blood-pressure-lowering treatments are warranted in all cerebral ischaemia, but recommendations about optimal drug regimen, including doses and when to start therapy, as well as target levels of LDL or BP to be achieved, are still debated. Revascularization procedures, including CEA or stenting, must be taken into consideration early after the event in patients with stroke or TIA related to carotid atherosclerotic disease.

## REFERENCES


1 **Murray CJ**, Lopez AD, Black R, Mathers CD, Shibuya K, Ezzati M, Salomon JA, Michaud CM, Walker N, Vos T. Global burden of disease 2005: call for collaborators. *Lancet* 2007; **370**: 109-110 [PMID: 17630021 DOI: 10.1016/S0140-6736(07)61064-2]

2 **Rothwell PM**, Coull AJ, Silver LE, Fairhead JF, Giles MF, Lovelock CE, Redgrave JN, Bull LM, Welch SJ, Cuthbertson FC, Binney LE, Gutnikov SA, Anslow P, Banning AP, Mant D, Mehta Z. Population-based study of event-rate, incidence, case fatality, and mortality for all acute vascular events in all arterial territories (Oxford Vascular Study). *Lancet* 2005; **366**: 1773-1783 [PMID: 16298214 DOI: 10.1016/S0140-6736(05)67702-1]

3 **Feigin VL**, Lawes CM, Bennett DA, Barker-Collo SL, Parag V. Worldwide stroke incidence and early case fatality reported in 56 population-based studies: a systematic review. *Lancet Neurol* 2009; **8**: 355-369 [PMID: 19233729 DOI: 10.1016/S1474-4422(09)70025-0]

4 **O'Donnell MJ**, Xavier D, Liu L, Zhang H, Chin SL, Rao-Melacini P, Rangarajan S, Islam S, Pais P, McQueen MJ, Mondo C, Damasceno A, Lopez-Jaramillo P, Hankey GJ, Dans AL, Yusoff K, Truelsen T, Diener HC, Sacco RL, Ryglewicz D, Czlonkowska A, Weimar C, Wang X, Yusuf S. Risk factors for ischaemic and intracerebral haemorrhagic stroke in 22 countries (the INTERSTROKE study): a case-control study. *Lancet* 2010; **376**: 112-123 [PMID: 20561675 DOI: 10.1016/S0140-6736(10)60834-3]

5 **Rothwell PM**, Coull AJ, Giles MF, Howard SC, Silver LE, Bull LM, Gutnikov SA, Edwards P, Mant D, Sackley CM, Farmer A, Sandercock PA, Dennis MS, Warlow CP, Bamford JM, Anslow P. Change in stroke incidence, mortality, case-fatality, severity, and risk factors in Oxfordshire, UK from 1981 to 2004 (Oxford Vascular Study). *Lancet* 2004; **363**: 1925-1933 [PMID: 15194251 DOI: 10.1016/S0140-6736(04)16405-2]

6 **Giles MF**, Rothwell PM. Risk of stroke early after transient ischaemic attack: a systematic review and meta-analysis. *Lancet Neurol* 2007; **6**: 1063-1072 [PMID: 17993293 DOI: 10.1016/S1474-4422(07)70274-0]

7 **Hong KS**, Yegiaian S, Lee M, Lee J, Saver JL. Declining stroke and vascular event recurrence rates in secondary prevention trials over the past 50 years and consequences for current trial design. *Circulation* 2011; **123**: 2111-2119 [PMID: 21536995 DOI: 10.1161/CIRCULATIONAHA.109.934786]

8 **Hankey GJ**, Jamrozik K, Broadhurst RJ, Forbes S, Burvill PW, Anderson CS, Stewart-Wynne EG. Long-term risk of first recurrent stroke in the Perth Community Stroke Study. *Stroke* 1998; **29**: 2491-2500 [PMID: 9836757 DOI: 10.1161/01.STR.29.12.2491]

9 **Burn J**, Dennis M, Bamford J, Sandercock P, Wade D, Warlow C. Long-term risk of recurrent stroke after a first-ever stroke. The Oxfordshire Community Stroke Project. *Stroke* 1994; **25**: 333-337 [PMID: 8303740 DOI: 10.1161/01.STR.25.2.333]

10 **Petty GW**, Brown RD, Whisnant JP, Sicks JD, O'Fallon WM, Wiebers DO. Survival and recurrence after first cerebral infarction: a population-based study in Rochester, Minnesota, 1975 through 1989. *Neurology* 1998; **50**: 208-216 [PMID: 9443482 DOI: 10.1212/WNL.50.1.208]

11 **Sacco RL**, Shi T, Zamanillo MC, Kargman DE. Predictors of mortality and recurrence after hospitalized cerebral infarction in an urban community: the Northern Manhattan Stroke Study. *Neurology* 1994; **44**: 626-634 [PMID: 8164815 DOI: 10.1212/WNL.44.4.626]

12 **Hillen T**, Coshall C, Tilling K, Rudd AG, McGovern R, Wolfe CD. Cause of stroke recurrence is multifactorial: patterns, risk factors, and outcomes of stroke recurrence in the South London Stroke Register. *Stroke* 2003; **34**: 1457-1463 [PMID: 12750544 DOI: 10.1161/01.STR.0000072985.24967.7F]

13 **Johnston SC**, Gress DR, Browner WS, Sidney S. Short-term prognosis after emergency department diagnosis of TIA. *JAMA* 2000; **284**: 2901-2906 [PMID: 11147987 DOI: 10.1001/jama.284.22.2901]

14 **Rothwell PM**, Warlow CP. Timing of TIAs preceding stroke: time window for prevention is very short. *Neurology* 2005; **64**: 817-820 [PMID: 15753415 DOI: 10.1212/01.WNL.0000152985.32732.EE]

15 **Hankey GJ**. Long-term outcome after ischaemic stroke/transient ischaemic attack. *Cerebrovasc Dis* 2003; **16** Suppl 1: 14-19

[PMID: 12698014 DOI: 10.1159/000069936]
16 **Lemmens R**, Smet S, Thijs VN. Clinical scores for predicting recurrence after transient ischemic attack or stroke: how good are they? *Stroke* 2013; **44**: 1198-1203 [PMID: 23482596 DOI: 10.1161/STROKEAHA.111.000141]
17 **Cucchiara BL**, Messe SR, Sansing L, MacKenzie L, Taylor RA, Pacelli J, Shah Q, Pollak ES, Kasner SE. D-dimer, magnetic resonance imaging diffusion-weighted imaging, and ABCD2 score for transient ischemic attack risk stratification. *J Stroke Cerebrovasc Dis* 2009; **18**: 367-373 [PMID: 19717021 DOI: 10.1016/j.jstrokecerebrovasdis.2009.01.006]
18 **Ay H**, Arsava EM, Johnston SC, Vangel M, Schwamm LH, Furie KL, Koroshetz WJ, Sorensen AG. Clinical- and imaging-based prediction of stroke risk after transient ischemic attack: the CIP model. *Stroke* 2009; **40**: 181-186 [PMID: 18948609 DOI: 10.1161/STROKEAHA.108.521476]
19 **Calvet D**, Touzé E, Oppenheim C, Turc G, Meder JF, Mas JL. DWI lesions and TIA etiology improve the prediction of stroke after TIA. *Stroke* 2009; **40**: 187-192 [PMID: 18988917 DOI: 10.1161/STROKEAHA.108.515817]
20 **Coutts SB**, Eliasziw M, Hill MD, Scott JN, Subramaniam S, Buchan AM, Demchuk AM. An improved scoring system for identifying patients at high early risk of stroke and functional impairment after an acute transient ischemic attack or minor stroke. *Int J Stroke* 2008; **3**: 3-10 [PMID: 18705908 DOI: 10.1111/j.1747-4949.2008.00182.x]
21 **Rothwell PM**, Algra A, Amarenco P. Medical treatment in acute and long-term secondary prevention after transient ischaemic attack and ischaemic stroke. *Lancet* 2011; **377**: 1681-1692 [PMID: 21571151 DOI: 10.1016/S0140-6736(11)60516-3]
22 **Rothwell PM**, Giles MF, Chandratheva A, Marquardt L, Geraghty O, Redgrave JN, Lovelock CE, Binney LE, Bull LM, Cuthbertson FC, Welch SJ, Bosch S, Alexander FC, Silver LE, Gutnikov SA, Mehta Z. Effect of urgent treatment of transient ischaemic attack and minor stroke on early recurrent stroke (EXPRESS study): a prospective population-based sequential comparison. *Lancet* 2007; **370**: 1432-1442 [PMID: 17928046 DOI: 10.1016/S0140-6736(07)61448-2]
23 **Lavallée PC**, Meseguer E, Abboud H, Cabrejo L, Olivot JM, Simon O, Mazighi M, Nifle C, Niclot P, Lapergue B, Klein IF, Brochet E, Steg PG, Lesèche G, Labreuche J, Touboul PJ, Amarenco P. A transient ischaemic attack clinic with round-the-clock access (SOS-TIA): feasibility and effects. *Lancet Neurol* 2007; **6**: 953-960 [PMID: 17928270 DOI: 10.1016/S1474-4422(07)70248-X]
24 **European Stroke Organisation (ESO) Executive Committee**; ESO Writing Committee. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. *Cerebrovasc Dis* 2008; **25**: 457-507 [PMID: 18477843 DOI: 10.1159/000131083]
25 **Furie KL**, Kasner SE, Adams RJ, Albers GW, Bush RL, Fagan SC, Halperin JL, Johnston SC, Katzan I, Kernan WN, Mitchell PH, Ovbiagele B, Palesch YY, Sacco RL, Schwamm LH, Wassertheil-Smoller S, Turan TN, Wentworth D. Guidelines for the prevention of stroke in patients with stroke or transient ischemic attack: a guideline for healthcare professionals from the american heart association/american stroke association. *Stroke* 2011; **42**: 227-276 [PMID: 20966421 DOI: 10.1161/STR.0b013e3181f7d043]
26 **Sandercock P**, Gubitz G, Foley P, Counsell C. Antiplatelet therapy for acute ischaemic stroke. *Cochrane Database Syst Rev* 2003; (**2**): CD000029 [PMID: 12804384]
27 **Chen ZM**, Sandercock P, Pan HC, Counsell C, Collins R, Liu LS, Xie JX, Warlow C, Peto R. Indications for early aspirin use in acute ischemic stroke : A combined analysis of 40 000 randomized patients from the chinese acute stroke trial and the international stroke trial. On behalf of the CAST and IST collaborative groups. *Stroke* 2000; **31**: 1240-1249 [PMID: 10835439 DOI: 10.1161/01.STR.31.6.1240]
28 **Lovett JK**, Coull AJ, Rothwell PM. Early risk of recurrence by subtype of ischemic stroke in population-based incidence studies. *Neurology* 2004; **62**: 569-573 [PMID: 14981172 DOI: 10.1212/01.WNL.0000110311.09970.83]
29 **Yusuf S**, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. *N Engl J Med* 2001; **345**: 494-502 [PMID: 11519503 DOI: 10.1056/NEJMoa010746]
30 **Geeganage CM**, Diener HC, Algra A, Chen C, Topol EJ, Dengler R, Markus HS, Bath MW, Bath PM. Dual or mono antiplatelet therapy for patients with acute ischemic stroke or transient ischemic attack: systematic review and meta-analysis of randomized controlled trials. *Stroke* 2012; **43**: 1058-1066 [PMID: 22282894 DOI: 10.1161/STROKEAHA.111.637686]
31 **Benavente OR**, Hart RG, McClure LA, Szychowski JM, Coffey CS, Pearce LA. Effects of clopidogrel added to aspirin in patients with recent lacunar stroke. *N Engl J Med* 2012; **367**: 817-825 [PMID: 22931315 DOI: 10.1056/NEJMoa1204133]
32 **Wang Y**, Wang Y, Zhao X, Liu L, Wang D, Wang C, Wang C, Li H, Meng X, Cui L, Jia J, Dong Q, Xu A, Zeng J, Li Y, Wang Z, Xia H, Johnston SC. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med* 2013; **369**: 11-19 [PMID: 23803136 DOI: 10.1056/NEJMoa1215340]
33 ClinicalTrials.gov. Platelet-oriented inhibition in new tia and minor ischemic stroke (POINT) trial. Available from: URL: http: //clinicaltrials.Gov/ct2/show/nct00991029?Term=nct00991029&rank=1 (accessed may 31, 2013)
34 ClinicalTrials.gov. Combination of clopidogrel and aspirin for prevention of early recurrence in acute attherothrombotic stroke (COMPRESS). Available from: URL: http: //clinicaltrials.Gov/ct2/show/nct00814268?Term=n-ct00814268&rank=1 (accessed may 31, 2013).
35 ClinicalTrials.gov. Triple antiplatelets for reducing dependency after ischaemic stroke (TARDIS). Available from: URL: http: //clinicaltrials.Gov/ct2/show/nct01661322?Term=tardis&rank=1 (accessed may 31, 2013)
36 **Jauch EC**, Saver JL, Adams HP, Bruno A, Connors JJ, Demaerschalk BM, Khatri P, McMullan PW, Qureshi AI, Rosenfield K, Scott PA, Summers DR, Wang DZ, Wintermark M, Yonas H. Guidelines for the early management of patients with acute ischemic stroke: a guideline for healthcare professionals from the American Heart Association/American Stroke Association. *Stroke* 2013; **44**: 870-947 [PMID: 23370205 DOI: 10.1161/STR.0b013e318284056a]
37 **Castilla-Guerra L**, Fernández-Moreno Mdel C. Update on the management of hypertension for secondary stroke prevention. *Eur Neurol* 2012; **68**: 1-7 [PMID: 22627064 DOI: 10.1159/000336836]
38 **Rashid P**, Leonardi-Bee J, Bath P. Blood pressure reduction and secondary prevention of stroke and other vascular events: a systematic review. *Stroke* 2003; **34**: 2741-2748 [PMID: 14576382 DOI: 10.1161/01.STR.0000092488.40085.15]
39 **Potter JF**, Robinson TG, Ford GA, Mistri A, James M, Chernova J, Jagger C. Controlling hypertension and hypotension immediately post-stroke (CHHIPS): a randomised, placebo-controlled, double-blind pilot trial. *Lancet Neurol* 2009; **8**: 48-56 [PMID: 19058760 DOI: 10.1016/S1474-4422(08)70263-1]
40 **Robinson TG**, Potter JF, Ford GA, Bulpitt CJ, Chernova J, Jagger C, James MA, Knight J, Markus HS, Mistri AK, Poulter NR. Effects of antihypertensive treatment after acute stroke in the Continue or Stop Post-Stroke Antihypertensives Collaborative Study (COSSACS): a prospective, randomised, open, blinded-endpoint trial. *Lancet Neurol* 2010; **9**: 767-775 [PMID: 20621562 DOI: 10.1016/S1474-4422(10)70163-0]
41 **Sandset EC**, Bath PM, Boysen G, Jatuzis D, Kõrv J, Lüders S, Murray GD, Richter PS, Roine RO, Terént A, Thijs V, Berge E. The angiotensin-receptor blocker candesartan for treatment of acute stroke (SCAST): a randomised, placebo-controlled, double-blind trial. *Lancet* 2011; **377**: 741-750 [PMID: 21316752 DOI: 10.1016/S0140-6736(11)60104-9]
42 **Alvarez-Sabín J**, Huertas R, Quintana M, Rubiera M, Delgado P, Ribó M, Molina CA, Montaner J. Prior statin use

may be associated with improved stroke outcome after tissue plasminogen activator. *Stroke* 2007; **38**: 1076-1078 [PMID: 17272777 DOI: 10.1161/01.STR.0000258075.58283.8f]
43 **Cimino M**, Gelosa P, Gianella A, Nobili E, Tremoli E, Sironi L. Statins: multiple mechanisms of action in the ischemic brain. *Neuroscientist* 2007; **13**: 208-213 [PMID: 17519364 DOI: 10.1177/1073858406297121]
44 **Flint AC**, Kamel H, Navi BB, Rao VA, Faigeles BS, Conell C, Klingman JG, Sidney S, Hills NK, Sorel M, Cullen SP, Johnston SC. Statin use during ischemic stroke hospitalization is strongly associated with improved poststroke survival. *Stroke* 2012; **43**: 147-154 [PMID: 22020026 DOI: 10.1161/STROKEAHA.111.627729]
45 **Kennedy J**, Hill MD, Ryckborst KJ, Eliasziw M, Demchuk AM, Buchan AM. Fast assessment of stroke and transient ischaemic attack to prevent early recurrence (FASTER): a randomised controlled pilot trial. *Lancet Neurol* 2007; **6**: 961-969 [PMID: 17931979 DOI: 10.1016/S1474-4422(07)70250-8]
46 **Squizzato A**, Romualdi E, Dentali F, Ageno W. Statins for acute ischemic stroke. *Cochrane Database Syst Rev* 2011; (**8**): CD007551 [PMID: 21833959]
47 **Briel M**, Schwartz GG, Thompson PL, de Lemos JA, Blazing MA, van Es GA, Kayikçioglu M, Arntz HR, den Hartog FR, Veeger NJ, Colivicchi F, Dupuis J, Okazaki S, Wright RS, Bucher HC, Nordmann AJ. Effects of early treatment with statins on short-term clinical outcomes in acute coronary syndromes: a meta-analysis of randomized controlled trials. *JAMA* 2006; **295**: 2046-2056 [PMID: 16670413 DOI: 10.1001/jama.295.17.2046]
48 **Gentile NT**, Seftchick MW, Huynh T, Kruus LK, Gaughan J. Decreased mortality by normalizing blood glucose after acute ischemic stroke. *Acad Emerg Med* 2006; **13**: 174-180 [PMID: 16436794 DOI: 10.1111/j.1553-2712.2006.tb01668.x]
49 **Williams LS**, Rotich J, Qi R, Fineberg N, Espay A, Bruno A, Fineberg SE, Tierney WR. Effects of admission hyperglycemia on mortality and costs in acute ischemic stroke. *Neurology* 2002; **59**: 67-71 [PMID: 12105309 DOI: 10.1212/WNL.59.1.67]
50 **Ribo M**, Molina CA, Delgado P, Rubiera M, Delgado-Mederos R, Rovira A, Munuera J, Alvarez-Sabin J. Hyperglycemia during ischemia rapidly accelerates brain damage in stroke patients treated with tPA. *J Cereb Blood Flow Metab* 2007; **27**: 1616-1622 [PMID: 17299452 DOI: 10.1038/sj.jcbfm.9600460]
51 **American Diabetes Association**. Standards of medical care in diabetes--2010. *Diabetes Care* 2010; **33** Suppl 1: S11-S61 [PMID: 20042772 DOI: 10.2337/dc10-S011]
52 **Lansberg MG**, O'Donnell MJ, Khatri P, Lang ES, Nguyen-Huynh MN, Schwartz NE, Sonnenberg FA, Schulman S, Vandvik PO, Spencer FA, Alonso-Coello P, Guyatt GH, Akl EA. Antithrombotic and thrombolytic therapy for ischemic stroke: Antithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. *Chest* 2012; **141**: e601S-e636S [PMID: 22315273 DOI: 10.1378/chest.11-2302]
53 **Algra A**, van Gijn J. Cumulative meta-analysis of aspirin efficacy after cerebral ischaemia of arterial origin. *J Neurol Neurosurg Psychiatry* 1999; **66**: 255 [PMID: 10071120 DOI: 10.1136/jnnp.66.2.255]
54 A randomized trial of anticoagulants versus aspirin after cerebral ischemia of presumed arterial origin. The Stroke Prevention in Reversible Ischemia Trial (SPIRIT) Study Group. *Ann Neurol* 1997; **42**: 857-865 [PMID: 9403477 DOI: 10.1002/ana.410420606]
55 **Mohr JP**, Thompson JL, Lazar RM, Levin B, Sacco RL, Furie KL, Kistler JP, Albers GW, Pettigrew LC, Adams HP, Jackson CM, Pullicino P. A comparison of warfarin and aspirin for the prevention of recurrent ischemic stroke. *N Engl J Med* 2001; **345**: 1444-1451 [PMID: 11794192 DOI: 10.1056/NEJMoa011258]
56 **Halkes PH**, van Gijn J, Kappelle LJ, Koudstaal PJ, Algra A. Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): randomised controlled trial. *Lancet* 2006; **367**: 1665-1673 [PMID: 16714187 DOI: 10.1016/S0140-6736(06)68734-5]
57 **Diener HC**, Cunha L, Forbes C, Sivenius J, Smets P, Lowenthal A. European Stroke Prevention Study. 2. Dipyridamole and acetylsalicylic acid in the secondary prevention of stroke. *J Neurol Sci* 1996; **143**: 1-13 [PMID: 8981292 DOI: 10.1016/S0022-510X(96)00308-5]
58 **Halkes PH**, Gray LJ, Bath PM, Diener HC, Guiraud-Chaumeil B, Yatsu FM, Algra A. Dipyridamole plus aspirin versus aspirin alone in secondary prevention after TIA or stroke: a meta-analysis by risk. *J Neurol Neurosurg Psychiatry* 2008; **79**: 1218-1223 [PMID: 18535024 DOI: 10.1136/jnnp.2008.143875]
59 **CAPRIE Steering Committee**. A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee. *Lancet* 1996; **348**: 1329-1339 [PMID: 8918275 DOI: 10.1016/S0140-6736(96)09457-3]
60 **Diener HC**, Bogousslavsky J, Brass LM, Cimminiello C, Csiba L, Kaste M, Leys D, Matias-Guiu J, Rupprecht HJ. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-controlled trial. *Lancet* 2004; **364**: 331-337 [PMID: 15276392 DOI: 10.1016/S0140-6736(04)16721-4]
61 **Bhatt DL**, Fox KA, Hacke W, Berger PB, Black HR, Boden WE, Cacoub P, Cohen EA, Creager MA, Easton JD, Flather MD, Haffner SM, Hamm CW, Hankey GJ, Johnston SC, Mak KH, Mas JL, Montalescot G, Pearson TA, Steg PG, Steinhubl SR, Weber MA, Brennan DM, Fabry-Ribaudo L, Booth J, Topol EJ. Clopidogrel and aspirin versus aspirin alone for the prevention of atherothrombotic events. *N Engl J Med* 2006; **354**: 1706-1717 [PMID: 16531616 DOI: 10.1056/NEJMoa060989]
62 **Thijs V**, Lemmens R, Fieuws S. Network meta-analysis: simultaneous meta-analysis of common antiplatelet regimens after transient ischaemic attack or stroke. *Eur Heart J* 2008; **29**: 1086-1092 [PMID: 18349026 DOI: 10.1093/eurheartj/ehn106]
63 **Sacco RL**, Diener HC, Yusuf S, Cotton D, Ounpuu S, Lawton WA, Palesch Y, Martin RH, Albers GW, Bath P, Bornstein N, Chan BP, Chen ST, Cunha L, Dahlöf B, De Keyser J, Donnan GA, Estol C, Gorelick P, Gu V, Hermansson K, Hilbrich L, Kaste M, Lu C, Machnig T, Pais P, Roberts R, Skvortsova V, Teal P, Toni D, Vandermaelen C, Voigt T, Weber M, Yoon BW. Aspirin and extended-release dipyridamole versus clopidogrel for recurrent stroke. *N Engl J Med* 2008; **359**: 1238-1251 [PMID: 18753638 DOI: 10.1056/NEJMoa0805002]
64 **Field TS**, Benavente OR. Current status of antiplatelet agents to prevent stroke. *Curr Neurol Neurosci Rep* 2011; **11**: 6-14 [PMID: 21104164 DOI: 10.1007/s11910-010-0162-y]
65 **Yip S**, Benavente O. Antiplatelet agents for stroke prevention. *Neurotherapeutics* 2011; **8**: 475-487 [PMID: 21761240 DOI: 10.1007/s13311-011-0060-2]
66 **Costa J**, Ferro JM, Matias-Guiu J, Alvarez-Sabin J, Torres F. Triflusal for preventing serious vascular events in people at high risk. *Cochrane Database Syst Rev* 2005; (**3**): CD004296 [PMID: 16034926]
67 **Kamal AK**, Naqvi I, Husain MR, Khealani BA. Cilostazol versus aspirin for secondary prevention of vascular events after stroke of arterial origin. *Cochrane Database Syst Rev* 2011; (**1**): CD008076 [PMID: 21249700]
68 ClinicalTrials.gov. Prevention of cardiovascular events in ischemic stroke patients with high risk of cerebral hemorrhage (PICASSO). Available from: URL: http: //clinicaltrials.Gov/ct2/show/study/nct01013532?Term=picasso&rank=1 (accessed may 31, 2013)
69 **Shinohara Y**, Nishimaru K, Sawada T, Terashi A, Handa S, Hirai S, Hayashi K, Tohgi H, Fukuuchi Y, Uchiyama S, Yamaguchi T, Kobayashi S, Kondo K, Otomo E, Gotoh F. Sarpogrelate-Aspirin Comparative Clinical Study for Efficacy and Safety in Secondary Prevention of Cerebral In-

farction (S-ACCESS): A randomized, double-blind, aspirin-controlled trial. *Stroke* 2008; **39**: 1827-1833 [PMID: 18388340 DOI: 10.1161/STROKEAHA.107.505131]
70 **Bousser MG**, Amarenco P, Chamorro A, Fisher M, Ford I, Fox KM, Hennerici MG, Mattle HP, Rothwell PM, de Cordoüe A, Fratacci MD. Terutroban versus aspirin in patients with cerebral ischaemic events (PERFORM): a randomised, double-blind, parallel-group trial. *Lancet* 2011; **377**: 2013-2022 [PMID: 21616527 DOI: 10.1016/S0140-6736(11)60600-4]
71 **Morrow DA**, Braunwald E, Bonaca MP, Ameriso SF, Dalby AJ, Fish MP, Fox KA, Lipka LJ, Liu X, Nicolau JC, Ophuis AJ, Paolasso E, Scirica BM, Spinar J, Theroux P, Wiviott SD, Strony J, Murphy SA. Vorapaxar in the secondary prevention of atherothrombotic events. *N Engl J Med* 2012; **366**: 1404-1413 [PMID: 22443427 DOI: 10.1056/NEJMoa1200933]
72 **Vanstreels L**, Molenberghs G, Voigt JU. Secondary stroke prevention: misguided by guidelines? *Acta Cardiol* 2012; **67**: 431-438 [PMID: 22997997]
73 **Alberts MJ**, Eikelboom JW, Hankey GJ. Antithrombotic therapy for stroke prevention in non-valvular atrial fibrillation. *Lancet Neurol* 2012; **11**: 1066-1081 [PMID: 23153406 DOI: 10.1016/S1474-4422(12)70258-2]
74 **Camm AJ**, Lip GY, De Caterina R, Savelieva I, Atar D, Hohnloser SH, Hindricks G, Kirchhof P. 2012 focused update of the ESC Guidelines for the management of atrial fibrillation: an update of the 2010 ESC Guidelines for the management of atrial fibrillation--developed with the special contribution of the European Heart Rhythm Association. *Europace* 2012; **14**: 1385-1413 [PMID: 22923145 DOI: 10.1093/europace/eus305]
75 **Pisters R**, Lane DA, Nieuwlaat R, de Vos CB, Crijns HJ, Lip GY. A novel user-friendly score (HAS-BLED) to assess 1-year risk of major bleeding in patients with atrial fibrillation: the Euro Heart Survey. *Chest* 2010; **138**: 1093-1100 [PMID: 20299623 DOI: 10.1378/chest.10-0134]
76 **Oldgren J**, Alings M, Darius H, Diener HC, Eikelboom J, Ezekowitz MD, Kamensky G, Reilly PA, Yang S, Yusuf S, Wallentin L, Connolly SJ. Risks for stroke, bleeding, and death in patients with atrial fibrillation receiving dabigatran or warfarin in relation to the CHADS2 score: a subgroup analysis of the RE-LY trial. *Ann Intern Med* 2011; **155**: 660-67, W204 [PMID: 22084332]
77 **McGrath ER**, Kapral MK, Fang J, Eikelboom JW, ó Conghaile A, Canavan M, O'Donnell MJ. Which risk factors are more associated with ischemic stroke than intracerebral hemorrhage in patients with atrial fibrillation? *Stroke* 2012; **43**: 2048-2054 [PMID: 22618379 DOI: 10.1161/STROKEAHA.112.654145]
78 Secondary prevention in non-rheumatic atrial fibrillation after transient ischaemic attack or minor stroke. EAFT (European Atrial Fibrillation Trial) Study Group. *Lancet* 1993; **342**: 1255-1262 [PMID: 7901582]
79 Adjusted-dose warfarin versus low-intensity, fixed-dose warfarin plus aspirin for high-risk patients with atrial fibrillation: Stroke Prevention in Atrial Fibrillation III randomised clinical trial. *Lancet* 1996; **348**: 633-638 [PMID: 8782752 DOI: 10.1016/S0140-6736(96)03487-3]
80 **Morocutti C**, Amabile G, Fattapposta F, Nicolosi A, Matteoli S, Trappolini M, Cataldo G, Milanesi G, Lavezzari M, Pamparana F, Coccheri S. Indobufen versus warfarin in the secondary prevention of major vascular events in nonrheumatic atrial fibrillation. SIFA (Studio Italiano Fibrillazione Atriale) Investigators. *Stroke* 1997; **28**: 1015-1021 [PMID: 9158644 DOI: 10.1161/01.STR.28.5.1015]
81 **Connolly S**, Pogue J, Hart R, Pfeffer M, Hohnloser S, Chrolavicius S, Pfeffer M, Hohnloser S, Yusuf S. Clopidogrel plus aspirin versus oral anticoagulation for atrial fibrillation in the Atrial fibrillation Clopidogrel Trial with Irbesartan for prevention of Vascular Events (ACTIVE W): a randomised controlled trial. *Lancet* 2006; **367**: 1903-1912 [PMID: 16765759 DOI: 10.1016/S0140-6736(06)68845-4]
82 **Connolly SJ**, Pogue J, Hart RG, Hohnloser SH, Pfeffer M, Chrolavicius S, Yusuf S. Effect of clopidogrel added to aspirin in patients with atrial fibrillation. *N Engl J Med* 2009; **360**: 2066-2078 [PMID: 19336502 DOI: 10.1056/NEJMoa0901301]
83 **Ansell J**, Hirsh J, Hylek E, Jacobson A, Crowther M, Palareti G. Pharmacology and management of the vitamin K antagonists: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines (8th Edition). *Chest* 2008; **133**: 160S-198S [PMID: 18574265]
84 **Albers GW**, Diener HC, Frison L, Grind M, Nevinson M, Partridge S, Halperin JL, Horrow J, Olsson SB, Petersen P, Vahanian A. Ximelagatran vs warfarin for stroke prevention in patients with nonvalvular atrial fibrillation: a randomized trial. *JAMA* 2005; **293**: 690-698 [PMID: 15701910 DOI: 10.1001/jama.293.6.690]
85 **Olsson SB**. Stroke prevention with the oral direct thrombin inhibitor ximelagatran compared with warfarin in patients with non-valvular atrial fibrillation (SPORTIF III): randomised controlled trial. *Lancet* 2003; **362**: 1691-1698 [PMID: 14643116 DOI: 10.1016/S0140-6736(03)14841-6]
86 **Connolly SJ**, Ezekowitz MD, Yusuf S, Eikelboom J, Oldgren J, Parekh A, Pogue J, Reilly PA, Themeles E, Varrone J, Wang S, Alings M, Xavier D, Zhu J, Diaz R, Lewis BS, Darius H, Diener HC, Joyner CD, Wallentin L. Dabigatran versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2009; **361**: 1139-1151 [PMID: 19717844 DOI: 10.1056/NEJMoa0905561]
87 **Eikelboom JW**, Wallentin L, Connolly SJ, Ezekowitz M, Healey JS, Oldgren J, Yang S, Alings M, Kaatz S, Hohnloser SH, Diener HC, Franzosi MG, Huber K, Reilly P, Varrone J, Yusuf S. Risk of bleeding with 2 doses of dabigatran compared with warfarin in older and younger patients with atrial fibrillation: an analysis of the randomized evaluation of long-term anticoagulant therapy (RE-LY) trial. *Circulation* 2011; **123**: 2363-2372 [PMID: 21576658 DOI: 10.1161/CIRCULATIONAHA.110.004747]
88 **Patel MR**, Mahaffey KW, Garg J, Pan G, Singer DE, Hacke W, Breithardt G, Halperin JL, Hankey GJ, Piccini JP, Becker RC, Nessel CC, Paolini JF, Berkowitz SD, Fox KA, Califf RM. Rivaroxaban versus warfarin in nonvalvular atrial fibrillation. *N Engl J Med* 2011; **365**: 883-891 [PMID: 21830957 DOI: 10.1056/NEJMoa1009638]
89 **Granger CB**, Alexander JH, McMurray JJ, Lopes RD, Hylek EM, Hanna M, Al-Khalidi HR, Ansell J, Atar D, Avezum A, Bahit MC, Diaz R, Easton JD, Ezekowitz JA, Flaker G, Garcia D, Geraldes M, Gersh BJ, Golitsyn S, Goto S, Hermosillo AG, Hohnloser SH, Horowitz J, Mohan P, Jansky P, Lewis BS, Lopez-Sendon JL, Pais P, Parkhomenko A, Verheugt FW, Zhu J, Wallentin L. Apixaban versus warfarin in patients with atrial fibrillation. *N Engl J Med* 2011; **365**: 981-992 [PMID: 21870978 DOI: 10.1056/NEJMoa1107039]
90 **Connolly SJ**, Eikelboom J, Joyner C, Diener HC, Hart R, Golitsyn S, Flaker G, Avezum A, Hohnloser SH, Diaz R, Talajic M, Zhu J, Pais P, Budaj A, Parkhomenko A, Jansky P, Commerford P, Tan RS, Sim KH, Lewis BS, Van Mieghem W, Lip GY, Kim JH, Lanas-Zanetti F, Gonzalez-Hermosillo A, Dans AL, Munawar M, O'Donnell M, Lawrence J, Lewis G, Afzal R, Yusuf S. Apixaban in patients with atrial fibrillation. *N Engl J Med* 2011; **364**: 806-817 [PMID: 21309657 DOI: 10.1056/NEJMoa1007432]
91 **Ruff CT**, Giugliano RP, Antman EM, Crugnale SE, Bocanegra T, Mercuri M, Hanyok J, Patel I, Shi M, Salazar D, McCabe CH, Braunwald E. Evaluation of the novel factor Xa inhibitor edoxaban compared with warfarin in patients with atrial fibrillation: design and rationale for the Effective aNticoaGulation with factor xA next GEneration in Atrial Fibrillation-Thrombolysis In Myocardial Infarction study 48 (ENGAGE AF-TIMI 48). *Am Heart J* 2010; **160**: 635-641 [PMID: 20934556 DOI: 10.1016/j.ahj.2010.06.042]
92 **Diener HC**, Connolly SJ, Ezekowitz MD, Wallentin L, Reilly PA, Yang S, Xavier D, Di Pasquale G, Yusuf S. Dabigatran compared with warfarin in patients with atrial fibrillation and previous transient ischaemic attack or stroke: a subgroup analysis of the RE-LY trial. *Lancet Neurol* 2010; **9**: 1157-1163

[PMID: 21059484 DOI: 10.1016/S1474-4422(10)70274-X]
93 **Hankey GJ**, Patel MR, Stevens SR, Becker RC, Breithardt G, Carolei A, Diener HC, Donnan GA, Halperin JL, Mahaffey KW, Mas JL, Massaro A, Norrving B, Nessel CC, Paolini JF, Roine RO, Singer DE, Wong L, Califf RM, Fox KA, Hacke W. Rivaroxaban compared with warfarin in patients with atrial fibrillation and previous stroke or transient ischaemic attack: a subgroup analysis of ROCKET AF. *Lancet Neurol* 2012; **11**: 315-322 [PMID: 22402056 DOI: 10.1016/S1474-4422(12)70042-X]
94 **Easton JD**, Lopes RD, Bahit MC, Wojdyla DM, Granger CB, Wallentin L, Alings M, Goto S, Lewis BS, Rosenqvist M, Hanna M, Mohan P, Alexander JH, Diener HC. Apixaban compared with warfarin in patients with atrial fibrillation and previous stroke or transient ischaemic attack: a subgroup analysis of the ARISTOTLE trial. *Lancet Neurol* 2012; **11**: 503-511 [PMID: 22572202 DOI: 10.1016/S1474-4422(12)70092-3]
95 **Diener HC**, Eikelboom J, Connolly SJ, Joyner CD, Hart RG, Lip GY, O'Donnell M, Hohnloser SH, Hankey GJ, Shestakovska O, Yusuf S. Apixaban versus aspirin in patients with atrial fibrillation and previous stroke or transient ischaemic attack: a predefined subgroup analysis from AVERROES, a randomised trial. *Lancet Neurol* 2012; **11**: 225-231 [PMID: 22305462 DOI: 10.1016/S1474-4422(12)70017-0]
96 **Miller CS**, Grandi SM, Shimony A, Filion KB, Eisenberg MJ. Meta-analysis of efficacy and safety of new oral anticoagulants (dabigatran, rivaroxaban, apixaban) versus warfarin in patients with atrial fibrillation. *Am J Cardiol* 2012; **110**: 453-460 [PMID: 22537354 DOI: 10.1016/j.amjcard.2012.03.049]
97 **Lip GY**, Larsen TB, Skjøth F, Rasmussen LH. Indirect comparisons of new oral anticoagulant drugs for efficacy and safety when used for stroke prevention in atrial fibrillation. *J Am Coll Cardiol* 2012; **60**: 738-746 [PMID: 22575324 DOI: 10.1016/j.jacc.2012.03.019]
98 **Schneeweiss S**, Gagne JJ, Patrick AR, Choudhry NK, Avorn J. Comparative efficacy and safety of new oral anticoagulants in patients with atrial fibrillation. *Circ Cardiovasc Qual Outcomes* 2012; **5**: 480-486 [PMID: 22787066 DOI: 10.1161/CIRCOUTCOMES.112.965988]
99 **Harenberg J**, Marx S, Diener HC, Lip GY, Marder VJ, Wehling M, Weiss C. Comparison of efficacy and safety of dabigatran, rivaroxaban and apixaban in patients with atrial fibrillation using network meta-analysis. *Int Angiol* 2012; **31**: 330-339 [PMID: 22801398]
100 **Mantha S**, Ansell J. An indirect comparison of dabigatran, rivaroxaban and apixaban for atrial fibrillation. *Thromb Haemost* 2012; **108**: 476-484 [PMID: 22740145 DOI: 10.1160/TH12-02-0093]
101 **Testa L**, Agnifili M, Latini RA, Mattioli R, Lanotte S, De Marco F, Oreglia J, Latib A, Pizzocri S, Laudisa ML, Brambilla N, Bedogni F. Adjusted indirect comparison of new oral anticoagulants for stroke prevention in atrial fibrillation. *QJM* 2012; **105**: 949-957 [PMID: 22771555 DOI: 10.1093/qjmed/hcs114]
102 **Baker WL**, Phung OJ. Systematic review and adjusted indirect comparison meta-analysis of oral anticoagulants in atrial fibrillation. *Circ Cardiovasc Qual Outcomes* 2012; **5**: 711-719 [PMID: 22912382 DOI: 10.1161/CIRCOUTCOMES.112.966572]
103 **Dentali F**, Riva N, Crowther M, Turpie AG, Lip GY, Ageno W. Efficacy and safety of the novel oral anticoagulants in atrial fibrillation: a systematic review and meta-analysis of the literature. *Circulation* 2012; **126**: 2381-2391 [PMID: 23071159 DOI: 10.1161/CIRCULATIONAHA.112.115410]
104 **PROGRESS Collaborative Group**. Randomised trial of a perindopril-based blood-pressure-lowering regimen among 6,105 individuals with previous stroke or transient ischaemic attack. *Lancet* 2001; **358**: 1033-1041 [PMID: 11589932 DOI: 10.1016/S0140-6736(01)06178-5]
105 **Schrader J**, Lüders S, Kulschewski A, Hammersen F, Plate K, Berger J, Zidek W, Dominiak P, Diener HC. Morbidity and Mortality After Stroke, Eprosartan Compared with Nitrendipine for Secondary Prevention: principal results of a prospective randomized controlled study (MOSES). *Stroke* 2005; **36**: 1218-1226 [PMID: 15879332 DOI: 10.1161/01.STR.0000166048.35740.a9]
106 **Yusuf S**, Diener HC, Sacco RL, Cotton D, Ounpuu S, Lawton WA, Palesch Y, Martin RH, Albers GW, Bath P, Bornstein N, Chan BP, Chen ST, Cunha L, Dahlöf B, De Keyser J, Donnan GA, Estol C, Gorelick P, Gu V, Hermansson K, Hilbrich L, Kaste M, Lu C, Machnig T, Pais P, Roberts R, Skvortsova V, Teal P, Toni D, VanderMaelen C, Voigt T, Weber M, Yoon BW. Telmisartan to prevent recurrent stroke and cardiovascular events. *N Engl J Med* 2008; **359**: 1225-1237 [PMID: 18753639 DOI: 10.1056/NEJMoa0804593]
107 **Yusuf S**, Teo KK, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P, Anderson C. Telmisartan, ramipril, or both in patients at high risk for vascular events. *N Engl J Med* 2008; **358**: 1547-1559 [PMID: 18378520 DOI: 10.1056/NEJMoa0801317]
108 **Yusuf S**, Teo K, Anderson C, Pogue J, Dyal L, Copland I, Schumacher H, Dagenais G, Sleight P. Effects of the angiotensin-receptor blocker telmisartan on cardiovascular events in high-risk patients intolerant to angiotensin-converting enzyme inhibitors: a randomised controlled trial. *Lancet* 2008; **372**: 1174-1183 [PMID: 18757085 DOI: 10.1016/S0140-6736(08)61242-8]
109 **Chobanian AV**, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; **289**: 2560-2572 [PMID: 12748199 DOI: 10.1001/jama.289.19.2560]
110 **Rothwell PM**. Limitations of the usual blood-pressure hypothesis and importance of variability, instability, and episodic hypertension. *Lancet* 2010; **375**: 938-948 [PMID: 20226991 DOI: 10.1016/S0140-6736(10)60309-1]
111 **Rothwell PM**, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, Sever PS, Poulter NR. Prognostic significance of visit-to-visit variability, maximum systolic blood pressure, and episodic hypertension. *Lancet* 2010; **375**: 895-905 [PMID: 20226988 DOI: 10.1016/S0140-6736(10)60308-X]
112 **Webb AJ**, Fischer U, Mehta Z, Rothwell PM. Effects of antihypertensive-drug class on interindividual variation in blood pressure and risk of stroke: a systematic review and meta-analysis. *Lancet* 2010; **375**: 906-915 [PMID: 20226989 DOI: 10.1016/S0140-6736(10)60235-8]
113 **Rothwell PM**, Howard SC, Dolan E, O'Brien E, Dobson JE, Dahlöf B, Poulter NR, Sever PS. Effects of beta blockers and calcium-channel blockers on within-individual variability in blood pressure and risk of stroke. *Lancet Neurol* 2010; **9**: 469-480 [PMID: 20227347 DOI: 10.1016/S1474-4422(10)70066-1]
114 **Webb AJ**, Rothwell PM. Effect of dose and combination of antihypertensives on interindividual blood pressure variability: a systematic review. *Stroke* 2011; **42**: 2860-2865 [PMID: 21817143 DOI: 10.1161/STROKEAHA.110.611566]
115 **Beckett NS**, Peters R, Fletcher AE, Staessen JA, Liu L, Dumitrascu D, Stoyanovsky V, Antikainen RL, Nikitin Y, Anderson C, Belhani A, Forette F, Rajkumar C, Thijs L, Banya W, Bulpitt CJ. Treatment of hypertension in patients 80 years of age or older. *N Engl J Med* 2008; **358**: 1887-1898 [PMID: 18378519 DOI: 10.1056/NEJMoa0801369]
116 **Bakris GL**, Sarafidis PA, Weir MR, Dahlöf B, Pitt B, Jamerson K, Velazquez EJ, Staikos-Byrne L, Kelly RY, Shi V, Chiang YT, Weber MA. Renal outcomes with different fixed-dose combination therapies in patients with hypertension at high risk for cardiovascular events (ACCOMPLISH): a prespecified secondary analysis of a randomised controlled trial. *Lancet* 2010; **375**: 1173-1181 [PMID: 20170948 DOI: 10.1016/S0140-6736(09)62100-0]
117 **Lewington S**, Whitlock G, Clarke R, Sherliker P, Emberson J, Halsey J, Qizilbash N, Peto R, Collins R. Blood cholesterol

and vascular mortality by age, sex, and blood pressure: a meta-analysis of individual data from 61 prospective studies with 55,000 vascular deaths. *Lancet* 2007; **370**: 1829-1839 [PMID: 18061058 DOI: 10.1016/S0140-6736(07)61778-4]

118 **Baigent C**, Blackwell L, Emberson J, Holland LE, Reith C, Bhala N, Peto R, Barnes EH, Keech A, Simes J, Collins R. Efficacy and safety of more intensive lowering of LDL cholesterol: a meta-analysis of data from 170,000 participants in 26 randomised trials. *Lancet* 2010; **376**: 1670-1681 [PMID: 21067804 DOI: 10.1016/S0140-6736(10)61350-5]

119 **Collins R**, Armitage J, Parish S, Sleight P, Peto R. Effects of cholesterol-lowering with simvastatin on stroke and other major vascular events in 20536 people with cerebrovascular disease or other high-risk conditions. *Lancet* 2004; **363**: 757-767 [PMID: 15016485 DOI: 10.1016/S0140-6736(04)15690-0]

120 **Amarenco P**, Bogousslavsky J, Callahan A, Goldstein LB, Hennerici M, Rudolph AE, Sillesen H, Simunovic L, Szarek M, Welch KM, Zivin JA. High-dose atorvastatin after stroke or transient ischemic attack. *N Engl J Med* 2006; **355**: 549-559 [PMID: 16899775 DOI: 10.1056/NEJMoa061894]

121 **Amarenco P**, Labreuche J. Lipid management in the prevention of stroke: review and updated meta-analysis of statins for stroke prevention. *Lancet Neurol* 2009; **8**: 453-463 [PMID: 19375663 DOI: 10.1016/S1474-4422(09)70058-4]

122 **Goldstein LB**, Amarenco P, Szarek M, Callahan A, Hennerici M, Sillesen H, Zivin JA, Welch KM. Hemorrhagic stroke in the Stroke Prevention by Aggressive Reduction in Cholesterol Levels study. *Neurology* 2008; **70**: 2364-2370 [PMID: 18077795 DOI: 10.1212/01.wnl.0000296277.63350.77]

123 **Furie KL**. High-dose statins should only be used in atherosclerotic strokes. *Stroke* 2012; **43**: 1994-1995 [PMID: 22581818 DOI: 10.1161/STROKEAHA.111.633339]

124 **Amarenco P**, Goldstein LB, Callahan A, Sillesen H, Hennerici MG, O'Neill BJ, Rudolph AE, Simunovic L, Zivin JA, Welch KM. Baseline blood pressure, low- and high-density lipoproteins, and triglycerides and the risk of vascular events in the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial. *Atherosclerosis* 2009; **204**: 515-520 [PMID: 18962621 DOI: 10.1016/j.atherosclerosis.2008.09.008]

125 **Amarenco P**, Goldstein LB, Szarek M, Sillesen H, Rudolph AE, Callahan A, Hennerici M, Simunovic L, Zivin JA, Welch KM. Effects of intense low-density lipoprotein cholesterol reduction in patients with stroke or transient ischemic attack: the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) trial. *Stroke* 2007; **38**: 3198-3204 [PMID: 17962589 DOI: 10.1161/STROKEAHA.107.493106]

126 ClinicalTrials.gov. Treat stroke to target (TST). Available from: URL: http: //clinicaltrials.Gov/ct2/show/nct01252875?Term=nct01252875&rank=1 (accessed may 31, 2013)

127 ClinicalTrials.gov. Optimal blood pressure and cholesterol targets for preventing recurrent stroke in hypertensive (ESH-CHL-SHOT). Available from: URL: http: //clinicaltrials.Gov/ct2/show/nct01563731?Term=esh-chl-shot&rank=1 (accessed may 31, 2013)

128 **LaRosa JC**, Grundy SM, Waters DD, Shear C, Barter P, Fruchart JC, Gotto AM, Greten H, Kastelein JJ, Shepherd J, Wenger NK. Intensive lipid lowering with atorvastatin in patients with stable coronary disease. *N Engl J Med* 2005; **352**: 1425-1435 [PMID: 15755765 DOI: 10.1056/NEJMoa050461]

129 ClinicalTrials.gov. Japan statin treatment against recurrent stroke (J-STARS). Available from: URL: http: //clinicaltrials.Gov/ct2/show/nct00221104?Term=nct00221104&rank=1 (accessed may 31, 2013)

130 Clofibrate and niacin in coronary heart disease. *JAMA* 1975; **231**: 360-381 [PMID: 1088963 DOI: 10.1001/jama.1975.03240160024021]

131 **Bloomfield Rubins H**, Davenport J, Babikian V, Brass LM, Collins D, Wexler L, Wagner S, Papademetriou V, Rutan G, Robins SJ. Reduction in stroke with gemfibrozil in men with coronary heart disease and low HDL cholesterol: The Veterans Affairs HDL Intervention Trial (VA-HIT). *Circulation* 2001; **103**: 2828-2833 [PMID: 11401940 DOI: 10.1161/01.CIR.103.23.2828]

132 **Overell JR**, Bone I, Lees KR. Interatrial septal abnormalities and stroke: a meta-analysis of case-control studies. *Neurology* 2000; **55**: 1172-1179 [PMID: 11071496 DOI: 10.1212/WNL.55.8.1172]

133 **Alsheikh-Ali AA**, Thaler DE, Kent DM. Patent foramen ovale in cryptogenic stroke: incidental or pathogenic? *Stroke* 2009; **40**: 2349-2355 [PMID: 19443800 DOI: 10.1161/STROKEAHA.109.547828]

134 **Homma S**, Sacco RL, Di Tullio MR, Sciacca RR, Mohr JP. Effect of medical treatment in stroke patients with patent foramen ovale: patent foramen ovale in Cryptogenic Stroke Study. *Circulation* 2002; **105**: 2625-2631 [PMID: 12045168 DOI: 10.1161/01.CIR.0000017498.88393.44]

135 **Mas JL**, Arquizan C, Lamy C, Zuber M, Cabanes L, Derumeaux G, Coste J. Recurrent cerebrovascular events associated with patent foramen ovale, atrial septal aneurysm, or both. *N Engl J Med* 2001; **345**: 1740-1746 [PMID: 11742048 DOI: 10.1056/NEJMoa011503]

136 **Meier B**, Frank B, Wahl A, Diener HC. Secondary stroke prevention: patent foramen ovale, aortic plaque, and carotid stenosis. *Eur Heart J* 2012; **33**: 705-13, 713a, 713b [PMID: 22422912]

137 **Kitsios GD**, Dahabreh IJ, Abu Dabrh AM, Thaler DE, Kent DM. Patent foramen ovale closure and medical treatments for secondary stroke prevention: a systematic review of observational and randomized evidence. *Stroke* 2012; **43**: 422-431 [PMID: 22180252 DOI: 10.1161/STROKEAHA.111.631648]

138 **Furlan AJ**, Reisman M, Massaro J, Mauri L, Adams H, Albers GW, Felberg R, Herrmann H, Kar S, Landzberg M, Raizner A, Wechsler L. Closure or medical therapy for cryptogenic stroke with patent foramen ovale. *N Engl J Med* 2012; **366**: 991-999 [PMID: 22417252 DOI: 10.1056/NEJMoa1009639]

139 **Carroll JD**, Saver JL, Thaler DE, Smalling RW, Berry S, MacDonald LA, Marks DS, Tirschwell DL. Closure of patent foramen ovale versus medical therapy after cryptogenic stroke. *N Engl J Med* 2013; **368**: 1092-1100 [PMID: 23514286 DOI: 10.1056/NEJMoa1301440]

140 **Meier B**, Kalesan B, Mattle HP, Khattab AA, Hildick-Smith D, Dudek D, Andersen G, Ibrahim R, Schuler G, Walton AS, Wahl A, Windecker S, Jüni P. Percutaneous closure of patent foramen ovale in cryptogenic embolism. *N Engl J Med* 2013; **368**: 1083-1091 [PMID: 23514285 DOI: 10.1056/NEJMoa1211716]

141 ClinicalTrials.gov. Patent foramen ovale closure or anticoagulants versus antiplatelet therapy to prevent stroke recurrence (CLOSE). Available from: URL: http: //clinicaltrials.Gov/ct2/show/nct00562289?Term=nct00562289&rank1 (accessed july 31, 2013)

142 ClinicalTrials.gov. Gore® helex® septal occluder / gore® septal occluder for patent foramen ovale (pfo) closure in stroke patients - the gore reduce clinical study (hlx 06-03). Available from: URL: http: //clinicaltrials.Gov/ct2/show/nct00730094?Term=nct00730094&rank1 (accessed july 31, 2013)

143 **Furlan AJ**, Jauss M. Patent foramen ovale and cryptogenic stroke: the hole story. *Stroke* 2013; **44**: 2676-2678 [PMID: 23908069 DOI: 10.1161/STROKEAHA.113.001676]

144 MRC European Carotid Surgery Trial: interim results for symptomatic patients with severe (70-99%) or with mild (0-29%) carotid stenosis. European Carotid Surgery Trialists' Collaborative Group. *Lancet* 1991; **337**: 1235-1243 [PMID: 1674060 DOI: 10.1016/0140-6736(91)92916-P]

145 North American Symptomatic Carotid Endarterectomy Trial Collaborators. Beneficial effect of carotid endarterectomy in symptomatic patients with high-grade carotid stenosis. *N Engl J Med* 1991; **325**: 445-453 [PMID: 1852179 DOI: 10.1056/NEJM199108153250701]

146 **Mayberg MR**, Wilson SE, Yatsu F, Weiss DG, Messina L, Hershey LA, Colling C, Eskridge J, Deykin D, Winn HR. Carotid endarterectomy and prevention of cerebral ischemia

in symptomatic carotid stenosis. Veterans Affairs Cooperative Studies Program 309 Trialist Group. *JAMA* 1991; **266**: 3289-3294 [PMID: 1960828 DOI: 10.1001/jama.1991.03470230047029]

147 Endovascular versus surgical treatment in patients with carotid stenosis in the Carotid and Vertebral Artery Transluminal Angioplasty Study (CAVATAS): a randomised trial. *Lancet* 2001; **357**: 1729-1737 [PMID: 11403808 DOI: 10.1016/S0140-6736(00)04893-5]

148 **Ringleb PA**, Allenberg J, Brückmann H, Eckstein HH, Fraedrich G, Hartmann M, Hennerici M, Jansen O, Klein G, Kunze A, Marx P, Niederkorn K, Schmiedt W, Solymosi L, Stingele R, Zeumer H, Hacke W. 30 day results from the SPACE trial of stent-protected angioplasty versus carotid endarterectomy in symptomatic patients: a randomised non-inferiority trial. *Lancet* 2006; **368**: 1239-1247 [PMID: 17027729 DOI: 10.1016/S0140-6736(06)69122-8]

149 **Yadav JS**, Wholey MH, Kuntz RE, Fayad P, Katzen BT, Mishkel GJ, Bajwa TK, Whitlow P, Strickman NE, Jaff MR, Popma JJ, Snead DB, Cutlip DE, Firth BG, Ouriel K. Protected carotid-artery stenting versus endarterectomy in high-risk patients. *N Engl J Med* 2004; **351**: 1493-1501 [PMID: 15470212 DOI: 10.1056/NEJMoa040127]

150 **Cambria RP**. Commentary on: Mas JL, Chatellier G, Beyssen B, et al. Endarterectomy versus stenting in patients with symptomatic severe carotid stenosis. N Engl J Med. 2006; 355: 1660-1671. *Perspect Vasc Surg Endovasc Ther* 2007; **19**: 201-203 [PMID: 17704496 DOI: 10.1177/1531003507301665]

151 **Brott TG**, Hobson RW, Howard G, Roubin GS, Clark WM, Brooks W, Mackey A, Hill MD, Leimgruber PP, Sheffet AJ, Howard VJ, Moore WS, Voeks JH, Hopkins LN, Cutlip DE, Cohen DJ, Popma JJ, Ferguson RD, Cohen SN, Blackshear JL, Silver FL, Mohr JP, Lal BK, Meschia JF. Stenting versus endarterectomy for treatment of carotid-artery stenosis. *N Engl J Med* 2010; **363**: 11-23 [PMID: 20505173 DOI: 10.1056/NEJMoa0912321]

152 **Bonati LH**, Lyrer P, Ederle J, Featherstone R, Brown MM. Percutaneous transluminal balloon angioplasty and stenting for carotid artery stenosis. *Cochrane Database Syst Rev* 2012; **9**: CD000515 [PMID: 22972047]

153 **Chimowitz MI**, Lynn MJ, Derdeyn CP, Turan TN, Fiorella D, Lane BF, Janis LS, Lutsep HL, Barnwell SL, Waters MF, Hoh BL, Hourihane JM, Levy EI, Alexandrov AV, Harrigan MR, Chiu D, Klucznik RP, Clark JM, McDougall CG, Johnson MD, Pride GL, Torbey MT, Zaidat OO, Rumboldt Z, Cloft HJ. Stenting versus aggressive medical therapy for intracranial arterial stenosis. *N Engl J Med* 2011; **365**: 993-1003 [PMID: 21899409 DOI: 10.1056/NEJMoa1105335]

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REVIEW

# Molecular diagnosis of autosomal recessive cerebellar ataxia in the whole exome/genome sequencing era

Christina Votsi, Kyproula Christodoulou

Christina Votsi, Kyproula Christodoulou, Neurogenetics Department, The Cyprus Institute of Neurology and Genetics, Nicosia 1683, Cyprus
Author contributions: Votsi C and Christodoulou K contributed equally to the design, drafting and revision of the manuscript and both approved the final version.
Correspondence to: Kyproula Christodoulou, PhD, Professor of the Cyprus School of Molecular Medicine, Head of the Neurogenetics Department, The Cyprus Institute of Neurology and Genetics, 6 International Airport Av., PO Box 23462, Nicosia 1683, Cyprus. roula@cing.ac.cy
Telephone: +357-22-392649 Fax: +357-22-392615
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## Abstract

Autosomal recessive cerebellar ataxias (ARCA) are a clinically and genetically heterogeneous group of rare neurodegenerative disorders characterized by autosomal recessive inheritance and an early age of onset. Progressive ataxia is usually the prominent symptom and is often associated with other neurological or additional features. ARCA classification still remains controversial even though different approaches have been proposed over the years. Furthermore, ARCA molecular diagnosis has been a challenge due to phenotypic overlap and increased genetic heterogeneity observed within this group of disorders. Friedreich's ataxia and ataxia telangiectasia have been reported as the most frequent and well-studied forms of ARCA. Significant progress in understanding the genetic etiologies of the ARCA has been achieved during the last 15 years. The methodological revolution that has been observed in genetics over the last few years has contributed significantly to the molecular diagnosis of rare diseases including the ARCAs. Development of high throughput technologies has resulted in the identification of new ARCA genes and novel mutations in known ARCA genes. Therefore, an improvement in the molecular diagnosis of ARCA is expected. Moreover, based on the fact that many patients still remain undiagnosed, additional forms of ataxia are expected to be identified. We hereby review the current knowledge on the ARCAs, focused on the genetic findings of the most common forms that were molecularly characterized before the whole exome/genome era, as well as the most recently described forms that have been elucidated with the use of these novel technologies. The significant contribution of whole-exome sequencing or whole-genome sequencing in the molecular diagnosis of ARCAs is discussed.

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**Key words:** Autosomal recessive cerebellar ataxia; Whole-exome sequencing; Whole-genome sequencing; Homozygosity mapping; Next generation sequencing

**Core tip:** Molecular diagnosis of autosomal recessive cerebellar ataxias (ARCA) is challenging due to clinical overlap and increased genetic heterogeneity. Although use of traditional techniques led to the identification of causative mutations in the past, the recent employment of novel technologies in this field, has initiated a new era in the molecular diagnosis of ARCA. Limitations such as small sized families, large numbers of candidate genes within mapped intervals and large sized genes hindered the timely discovery of ARCA genes using conventional Sanger sequencing. ARCA gene discovery and molecular diagnosis should be achievable at a much faster rate through the use of whole-genome or whole-exome sequencing technologies.

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## INTRODUCTION

Autosomal recessive cerebellar ataxias (ARCA) constitute a subgroup of the hereditary cerebellar ataxias[1]. They are clinically and genetically heterogeneous neurodegenerative disorders characterized by autosomal recessive inheritance and usually a clinical onset before the age of 20. Both the central and the peripheral nervous systems may be involved, as well as other systems and organs in rare cases[1,2]. In most cases the cerebellum, the spinocerebellar tract and/or the sensory tracts of the spinal cord are primarily affected[3]. Therefore, progressive ataxia is the main symptom and is often associated with other neurological or extra-neurological signs.

This group encompasses many rare diseases; Friedreich's ataxia and ataxia telangiectasia being the most frequent forms[2-4]. Their prevalence has been estimated to be 7 in 100000 inhabitants[5] with considerable variability observed in different geographic regions[3,6,7]. Many classifications have been proposed using different criteria and this issue still remains controversial. The first clinical classification of inherited ataxias, also encompassing ARCA, was proposed by Harding in 1983 and was based on the age of onset and pathological mechanisms[8,9]. Through the next decade, genetic studies led to new insights and thus additional criteria were used in some classifications. Koenig[10] proposed a classification of ARCA based on topographical and pathophysiological criteria, while in the next year the group of Filla proposed a pathogenic classification of the hereditary ataxias[11]. More recently, Palau and Espinos proposed a classification of ARCA that is based on inheritance patterns and natural history of the clinical symptoms[1].

Through the past 15 years, significant progress has been made in improving our understanding of the genetic etiology of the ARCA. Currently, more than 30 genes have been associated with ARCA (Table 1) and many more are expected to be discovered since the genetic causes still remain unknown, for a large number of patients/families. The identified ARCA genes are involved in variable cellular processes such as mitochondrial energy generation, DNA repair, transcription, RNA processing, protein folding and ion channels[12].

Despite the existence of different classification schemes and the significant number of already identified genes, diagnosis of ARCA remains a challenge due to their vast clinical variability and genetic heterogeneity[13-15]. The methodological revolution of genetics observed over the last years contributed significantly to the proper diagnosis of ARCA. The recently developed next-generation sequencing (NGS) technologies, whole-exome sequencing (WES)/whole-genome sequencing (WGS), or the strategy of using homozygosity linkage mapping combined with NGS, proved to be efficient for the identification of novel genes associated with rare diseases. In comparison to the traditional methods widely used in the past for gene identification, the new techniques are easier, cost and time-efficient since they offer the opportunity to screen the whole-genome or the coding part of the genome in one experiment[14]. Limitations such as locus heterogeneity, small family size and existence of numerous candidate genes within a mapped region are no longer an obstacle[16]. Although relatively new, these technologies proved to be powerful methods for gene identification in rare neurological disorders such as ataxia; 7 new genes were identified in the past 3 years. Moreover, exome sequencing is gradually used as a screening tool thus enabling the identification of mutations in already known genes[17-21].

We hereby provide a review focused on the molecular diagnosis of ARCA with more emphasis on the recent discoveries observed in the whole exome/genome sequencing era.

## MOLECULAR DIAGNOSIS OF AUTOSOMAL RECESSIVE CEREBELLAR ATAXIAS WITH THE CONVENTIONAL METHODS

The most common ARCA forms that have been molecularly diagnosed through the use of traditional methods as well as the most common mutations identified are outlined below. If an affected individual is clinically suspected of having a specific type of the following ARCA, then investigation could begin with targeted mutation analysis.

### *Degenerative ataxias*

**Friedreich ataxia:** Friedreich ataxia is the most frequent inherited ataxia in the Caucasian population[1] and the most common form of degenerative recessive ataxia with an estimated prevalence of 1-2/50000[22-24]. The disease has been associated with mutations in the *FXN* gene located on chromosome 9q13[25-27]. A homozygous GAA repeat expansion within the first intron of the gene is the causative mutation for 95% of the cases[28], while the rest 5% of the cases are compound heterozygous for a GAA expansion and a point mutation in the *FXN* gene[29]. The expansion mutation leads to an impairment of transcription and further to a significant reduction in the expression of the encoded protein, frataxin. Although the function of frataxin has not been fully elucidated, its role in cellular iron homeostasis has been established. The deficiency caused by the expansion mutation results in mitochondrial dysfunction and oxidative damage[24,30].

Friedreich ataxia is considered as the first candidate for genetic testing of patients with ARCA in most populations. Specific polymerase chain reaction (PCR)-based molecular tests for the GAA repeat expansion are available in many laboratories[31-33]. Suspected compound heterozygote cases may be further investigated by Sanger sequencing of the *FXN* gene coding regions.

**Mitochondrial recessive ataxia syndrome:** Mitochondrial recessive ataxia syndrome is the most frequent juvenile- or adult-onset type of ataxia in Finland[34]. The disease has been associated with mutations in the *POLG* gene, encoding polymerase γ which is implicated in

**Table 1 Autosomal recessive cerebellar ataxias**

| Type | Gene/protein | Gene OMIM# | Gene locus |
|---|---|---|---|
| **Degenerative ataxias** | | | |
| Friedreich's ataxia | *FXN*/Frataxin | *606829 | 9q13 |
| SCAR9 | *ADCK3*/aarF domain-containing protein kinase 3 | *606980 | 1q42.13 |
| MIRAS | *POLG*/Polymerase γ | *174763 | 15q25 |
| IOSCA (MTDPS7) | *C10orf2*/Twinkle | *606075 | 10q24 |
| Marinesco-Sjögren syndrome | *SIL1*/BiP associated protein | *608005 | 5q31 |
| Charlevoix-Saguenay spastic ataxia | *SACS*/Sacsin | *604490 | 13q12 |
| EOCARR (EOCA) | - | - | 13q11-12 |
| **Ataxias with DNA repair defects** | | | |
| AOA1 | *APTX*/Aprataxin | *606350 | 9p13.3 |
| AOA2 (SCAR1) | *SETX*/Senataxin | *608465 | 9q34 |
| AOA3 | *PIK3R5*/Phosphoinositide 3-kinase regulatory subunit 5 | *611317 | 17p13.1 |
| AT | *ATM*/ATM | *607585 | 11q22.3 |
| ATLD | *MRE11A*/MRE11 | *600814 | 11q21 |
| SCAN1 | *TDP1*/Tyrosyl DNA phosphodiesterase Ⅰ | *607198 | 14q31-q32 |
| **Congenital ataxias** | | | |
| JBTS1 | *INPP5E*/Phosphatidylinositol polyphosphate 5-phosphatase type Ⅳ | *613037 | 9q34.3 |
| JBTS2 | *TMEM216*/transmembrane protein 216 | *613277 | 11p12-q13.3 |
| JBTS3 | *AHI1*/Jouberin | *608894 | 6q23.3 |
| JBTS4 | *NPHP1*/Nephrocystin-1 | *607100 | 2q13 |
| JBTS5 | *CEP290*/Nephrocystin-6 | *610142 | 12q21.3 |
| JBTS6 | *TMEM67*/Meckelin | *609884 | 8q22.1 |
| JBTS7 | *RPGRIP1L*/Nephrocystin-8 | *610937 | 16q12.2 |
| Cayman ataxia | *ATCAY*/Caytaxin | *608179 | 19p13.3 |
| **Metabolic ataxias** | | | |
| AVED (VED) | α-TTP/α-tocopherol transfer protein | *600415 | 8q13.1-q13.3 |
| ABL | *MTP*/Microsomal triglyceride transfer protein | *157147 | 4q22-24 |
| CTX | *CYP27*/Sterol 27-hydroxylase | *606530 | 2q33-qter |
| Refsum's disease | *PHYH*/Phytanoyl-CoA hydrolase | *602026 | 10pter-p11.2 |
| | *PEX7*/Peroxin 7 | *601757 | 6q22-q24 |
| Metachromatic leucodystrophy | *ARSA*/Arylsulfatase 1 | *607574 | 22q13.31-qter |
| Niemann Pick disease type C | *NPC1*/NPC1 protein | *607623 | 18q11-q12 |
| GM1 gangliosidosis | *GLB1*/Beta-galactosidase | *611458 | 3p21.33 |
| GM2 gangliosidosis | *HEXA*/Hexosaminidase A | *606869 | 15q23-24 |
| Wilson disease | *ATP7B*/ATPase Cu transporting beta-polypeptide | *606882 | 13q14.3 |
| Aceruloplasminemia | *CP*/Ceruloplasmin | *117700 | 3q23-q24 |
| CHAC | *VPS13A*/Chorein | *605978 | 9q21 |
| **Other recently identified types** | | | |
| SCAR8 | *SYNE1*/Syne-1 | *608441 | 6q25.1-q25.2 |
| Rundataxin-related ataxia | *KIAA0226*/Rundataxin | *613516 | 3q27.3-qter |
| **Novel types identified by the use of next-generation sequencing** | | | |
| SCAR10 | *ANO10*/Anoctamin 10 | *613726 | 3p22.1 |
| SCAR11 | *SYT14*/Synaptotagmin XIV | *610949 | 1q32.2 |
| SCAR7 | *TPP1*/tripeptidyl-peptidase 1 enzyme | *607998 | 11p15.4 |
| Autosomal-recessive cerebellar ataxia with spasticity | *GBA2*/β-glucosidase 2 | *609471 | 9p13.3 |
| SCAR13 | *GRM1*/Metabotropic glutamate receptor 1 | *604473 | 6q24.3 |

SCAR7: Spinocerebellar ataxia autosomal recessive type 7; SCAR8: Spinocerebellar ataxia autosomal recessive type 8; SCAR9: Spinocerebellar ataxia autosomal recessive type 9; SCAR10: Spinocerebellar ataxia autosomal recessive type 10; SCAR11: Spinocerebellar ataxia autosomal recessive type 11; SCAR13: Spinocerebellar ataxia autosomal recessive type 13; MIRAS: Mitochondrial recessive ataxic syndrome; IOSCA: Infantile-onset spinocerebellar ataxia; AOA: Ataxias with oculomotor apraxia; AT: Ataxia telangiectasia; ATLD: Ataxia-telangiectasia-like disorder; SCAN1: Spinocerebellar ataxia with axonal neuropathy; CTX: Cerebrotendinous xanthomatosis; CHAC: Chorea-acanthocytosis; JBTS: Joubert syndrome; AVED: Ataxia with isolated vitamin E deficiency; ABL:Abetalipoproteinemia.

mtDNA replication and repair. The most common mutation identified thus far is the c.2243G > C [p.Trp748Ser] associated with the c.3428A > G [p.Glu1143Gly] mutation which is probably a polymorphism with a modifying role. Another frequent mutation is the c.1399G > A [p.Ala467Thr]. Homozygous or compound heterozygous patients for the three mutations have been reported[34-36]. The presence of the three mutations may be molecularly diagnosed by PCR and solid-phase minisequencing while a Real time PCR assay has been proposed for identifying the presence of the c.1399G > A mutation[34].

**Infantile onset spinocerebellar ataxia:** Infantile onset spinocerebellar ataxia is a rare disorder manifesting at a very early age and was first described in Finland. It has been associated with mutations in the *C10orf2* gene on chromosome 10q24 that encodes Twinkle, a mtDNA specific helicase and Twinky, a rarer splice variant[37]. The most com-

mon mutation identified is c.1523A > G [p.Tyr508Cys]. Mutations in this gene may be detected either by direct sequencing or by solid-phase minisequencing.

**Autosomal recessive spastic ataxia of Charlevoix-Saguenay:** Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is an early childhood onset disease characterized by spastic gait ataxia. It was first described in families from the Charlevoix-Saguenay region of northeastern Quebec and for many years it was thought to be restricted there. It has been mapped to chromosome 13q11 and causative mutations have been identified in the *SACS* gene[38] that encodes sacsin, a chaperone protein. Almost 96% of the molecularly diagnosed individuals from this region are homozygote or compound heterozygote for the two founder mutations c.8844delT [p.Pro2948fs] and c.7504C > T [p.Arg2502*] (originally reported as 6594delT and 5254C > T) identified by sequence-based analyses[39,40]. More than 100 other pathogenic mutations in individuals outside Quebec were identified since mutation analysis became available, demonstrating that mutations in SACS are a frequent cause of the disease worldwide[41-43]. Molecular genetic testing is usually performed by targeted mutation analysis, by direct sequencing of all coding exons and their flanking intronic sequences and by deletion/duplication analysis using the multiplex ligation-dependent probe amplification method[44].

**Marinesco-Sjögren syndrome:** Marinesco-Sjögren syndrome is a rare disorder with onset in infancy or childhood. Homozygosity mapping in two large consanguineous families of Turkish and Norwegian origin mapped the disease gene on chromosome 5q31[45]. Causative mutations have been identified in patients of different ethnic origin, in the *SIL1* gene encoding a protein that acts as a nucleotide exchange factor for the heat-shock protein 70 chaperone the 78-kDa glucose-regulated protein, mainly located at the endoplastic reticulum[46-48]. Molecular genetic testing is performed by direct sequencing of all coding exons and their flanking intronic sequences.

**Spinocerebellar ataxia autosomal recessive type 9:** Spinocerebellar ataxia autosomal recessive type 9 (SCAR9), also known as autosomal recessive cerebellar ataxia type 2 (ARCA2) refers to ARCA associated with muscle coenzyme $Q_{10}$ deficiency[49]. Homozygosity mapping (GeneChip Human Mapping 10K 2.0 Xba Array, Affymetrix) in a large consanguineous Algerian family mapped the disease gene to chromosome 1q41-q42. *ADCK3* gene mutations have been identified by direct sequencing in this family, in additional families and also in sporadic patients[49-51]. The ADCK3 protein is a mitochondrial ancestral kinase which is involved in CoQ10 synthesis[49].

### *Ataxias with DNA repair defects*

**ARCA with oculomotor apraxia:** ARCA with oculomotor apraxia (OMA), known as ataxias with oculomotor apraxia (AOA), are characterized by cerebellar ataxia accompanied by ophthalmological and neurological symptoms[52,53]. Five distinct genetic forms have thus far been identified: ataxia-telangiectasia (A-T), ataxia-telangiectasia-like disorder (ATLD), ataxia with oculomotor apraxia type 1 (AOA1), ataxia with oculomotor apraxia type 2 (AOA2) and ataxia with oculomotor apraxia type 3 (AOA3)[54].

(1) Ataxia telangiectasia: Ataxia telangiectasia is the second most common ARCA after Friedreich ataxia. It presents in early childhood with progressive cerebellar ataxia, while later ataxia may be accompanied by a multitude of symptoms such as choreathetosis, dystonia, telangiectasia that is the guiding diagnostic feature, immunodeficiency, cancer predisposition, sinopulmonary infection, immunosensitivity and radiosensitivity[1,14]. The disease gene *ATM* is located on chromosome 11q23[55,56]. It is one of the largest genes, comprising 66 exons and encodes a member of the phosphoinositol-3 kinase family of proteins that are involved in cell cycle checkpoint control and the DNA repair through phosphorylation of substrates[1]. More than 500 mutations have been identified, that are positioned throughout all the coding exons of the gene[14]. Due to the large size of the gene, molecular diagnosis with the traditional methods has been difficult. A multiplex scanning method, detection of virtually all mutations-SSCP has been proposed however with reduced sensitivity[57]. The newly developed NGS technologies are promising for the diagnosis of diseases such as A-T with associated mutations in large genes.

(2) Ataxia-telangiectasia-like disorder: Ataxia-telangiectasia-like disorder (ATLD) is a very rare disorder that resembles A-T in its clinical phenotype[58] with absence of telangiectases and immune deficiency. It has been mapped to chromosome 11q21 and a small number of causative mutations have been identified in the *MRE11A* gene[59-61]. The encoded protein interacts with RAD50 and NBS1 thus forming the core of the MRN (MRE11-RAD50-NBS1) complex which is involved in DNA repair pathways, in DNA recombination and in multiple cell-cycle checkpoints control[60].

(3) Ataxia with oculomotor apraxia type 1: Ataxia with oculomotor apraxia type 1 (AOA1) was first described in Portuguese and Japanese families. The disease gene has been mapped to chromosome 9p13.3[62] and mutations in the *APTX* gene have been identified[63]. The encoded protein aprataxin, localizes to the nucleus[64] and the mitochondria[65] and it is involved in the nuclear and the mtDNA repair machinery. AOA1 is the most frequent type of ARCA in Japan and the second most frequent in Portugal after Friedreich ataxia. The insertion c.689insT and the missense c.617C > T [p.Pro206Leu] mutations, are more common in the Japanese and the nonsense c.837G > A [p.W279X] mutation is more common in Portuguese patients. More than 20 mutations including missense/nonsense or splice site mutations, small dele-

tions/insertions and gross deletions have been reported thus far in patients of different ethnicities, thus expanding the presence of AOA1 in other countries outside Japan and Portugal[3,66-70].

(4) Ataxia with oculomotor apraxia type 2: Ataxia with oculomotor apraxia type 2 (AOA2) is also referred to as autosomal recessive spinocerebellar ataxia 1 (SCAR1) since oculomotor apraxia is an occasional finding, not present in all the patients. It is the second most frequent recessive ataxia in Europe[4] and is usually (in 96% of cases) accompanied by a characteristic laboratory finding, the elevation of serum α-fetoprotein. AOA2 usually presents within the second decade of life and has a higher mean age of onset compared to AOA1 and A-T[52]. The disease gene has been mapped to chromosome 9q34 and later on mutations in the *SETX* gene were identified[71]. SETX is a large gene of 26 exons and encodes senataxin, a nuclear protein, which contains at its C terminus a classic seven-motif domain found in the superfamily 1 of helicases. The protein is involved in the response to oxidative stress[53]. Acting as an RNA/DNA helicase, senataxin might have a significant role in the DNA repair pathway, in the splicing machinery[71] and also in the processing of noncoding RNAs[52]. Even though the *SETX* gene cannot be easily analysed by routine sequencing due to its size, several patients have been studied with this method and more than 75 different mutations have been identified thus far worldwide[52,72,73] including missense, nonsense and splice site mutations, as well as small/gross deletions or insertions[74].

(5) Ataxia with oculomotor apraxia type 3: Ataxia with oculomotor apraxia type 3 has more recently been described in a consanguineous Saudi Arabian family with four affected individuals that present with a similar phenotype to AOA2. Elevated serum α-fetoprotein levels were identified in all the patients and 2 siblings had OMA. The homozygous missense c.1885C > T [p.Pro629Ser] mutation in exon 12 of the *PIK3R5* gene has been associated with the disease in this family[54]. *PIK3R5* is located on chromosome 17p12-p13. The encoded protein is a p101 regulatory subunit that interacts with class 1B PI3K which is involved in cell survival and growth as well as in metabolism, immune and cardiac functions. Even though it is registered as rs61761068 in db-single-nucleotide polymorphism (dbSNP), frequencies of the mutant allele in other populations indicate that it has only been detected in a heterozygous state, most likely representing non-affected carriers of the mutation[54].

**Spinocerebellar ataxia with axonal neuropathy:** Spinocerebellar ataxia with axonal neuropathy is a related syndrome to the AOA group. It is characterized by the co-occurrence of cerebellar ataxia and axonal neuropathy. A single locus on chromosome 14q31-q32 has been associated with the disease and a missense mutation (c.1478A > G [p.His493Arg]) in the *TDP1* gene has been described in a large Saudi Arabian family[75]. The encoded protein tyrosyl-DNA phosphodiesterase 1 is a member of the DNA single-strand break repair complex and plays a significant role in the DNA repair pathway[75,76].

### *Congenital and metabolic ataxias*

Other ARCA are classified under the subgroups of congenital and metabolic ataxias (Table 1). Associated genes and mutations have been identified and molecular genetic testing by targeted mutation analysis or direct sequencing of the appropriate gene may be performed in ARCA patients based on the clinical picture. Moreover, in some cases of metabolic ataxias, biochemical analysis on specific available markers could precede and orientate the genetic testing.

**Congenital ataxias:** Congenital ataxias that have been associated with specific chromosomal loci and genes include the Joubert syndrome and the Cayman ataxia. Joubert syndrome has thus far been associated with seven loci (JBTS1, JBTS2, JBTS3, JBTS4, JBTS5, JBTS6, and JBTS7) and mutations have been identified in the seven *INPP5E*[77], *TMEM216*[78], *AHI1*[79], *NPHP1*[80], *CEP290*[81], *TMEM67*[82] and *RPGRIP1L*[83] genes respectively (Table 1). The Cayman ataxia has been described in an isolated population of the Cayman Island. A single locus on chromosome 19p13.3 has been associated with the disease and causative mutations in the *ATCAY* gene have thus far been identified[84].

**Metabolic ataxias:** The subgroup of metabolic ataxias includes disorders characterized by progressive ataxia, recurrent ataxia and ataxia as a minor feature. Progressive ataxia includes ataxia with isolated vitamin E deficiency, abetalipoproteinemia, cerebrotendinous xanthomatosis and Refsum disease[1]. Intermittent or minor ataxia includes: metachromatic leukodystrophy (*ARSA* gene)[85,86], Niemann-Pick type C 1 (*NPC1* gene)[87], GM1 (*GLB1* gene)[88,89] and GM2 (*HEXA* gene)[90,91] gangliosidosis, Wilson's disease (*ATP7B* gene)[92-94], aceruloplasminaemia (*CP* gene)[95] and Chorea-acanthocytosis (*VPS13A* gene)[96].

(1) Ataxia with isolated vitamin E deficiency: Ataxia with isolated vitamin E deficiency has a similar clinical picture with Friedreich ataxia. It is characterized by a biochemical abnormality, the very low plasma level of vitamin E and hence the low level of the α-tocopherol in the serum. The disease is caused by mutations in the α-*TTP* gene, encoding the α-tocopherol transfer protein which is responsible for the transfer of vitamin E to the nascent very low-density lipoproteins[97]. Diagnosis may include the finding of low serum vitamin E values in the absence of malabsorption[1] and further by performing genetic testing.

(2) Abetalipoproteinemia: Abetalipoproteinemia is characterized by deficiency of the liposoluble vitamin E, the low levels of cholesterol and the absence of the low-density lipoproteins. The clinical features comprise the

malabsorption syndrome, the pigmentary degeneration of the retina and progressive ataxic neuropathy. Moreover, patients show a characteristic deformation of their red cells called acanthocytosis[98]. The disease is caused by mutations in the *MTP* gene which encodes the microsomal triglyceride transfer protein catalyzing the transport of triglyceride, cholesteryl ester and phospholipid between phospholipid surfaces[99].

(3) Cerebrotendinous xanthomatosis: Cerebrotendinous xanthomatosis is a sterol storage disorder, characterized by abnormal accumulation of cholesterol and cholestanol in neural and other tissues, thus causing tendon xanthomas, premature atherosclerosis, cataracts and neurological dysfunction which is manifested as cerebellar ataxia, dementia and spinal cord paresis[100]. The disease gene has been mapped to chromosome 2q33-qter and is caused by mutations in the *CYP27* gene have been identified. *CYP27* encodes sterol 27-hydroxylase, a mitochondrial cytochrome P450 enzyme that has an important role in cholesterol and bile acid metabolism[100,101].

(4) Refsum's disease: Refsum's disease is caused by defective peroxisomal alpha-oxidation of phytanic acid, thus leading to its accumulation in blood and other tissues including neurons. It is characterized mainly by progressive retinitis pigmentosa, peripheral neuropathy and cerebellar ataxia. Other additional features include anosmia, deafness, cardiomyopathy, ichthyosis and skeletal abnormalities. The disorder is genetically heterogeneous since it has been mapped to two different loci. In most families, linkage was established to the first identified locus on chromosome 10pter-p11.2. Causative mutations have been found in the *PHYH* (or *PAHX*) gene[102,103] encoding the phytanoyl-CoA hydroxylase enzyme that is involved in the catabolic pathway of phytanic acid. In a subset of patients mutations in the *PAHX* gene have been excluded. Further linkage analysis of these families led to mapping of a second locus on chromosome 6q22-24. In this subgroup of patients causative mutations have been identified in the *PEX7* gene encoding the peroxin 7 protein which is implicated in the import of matrix proteins into the peroxisome[104].

### *Other known ARCA types*

The following ARCA forms have been reported more recently that have not been included in the classification scheme described by Palau and Espinos (2006).

**Spinocerebellar ataxia autosomal recessive type 8:** Spinocerebellar ataxia autosomal recessive type 8 (SCAR8), also known as autosomal recessive cerebellar ataxia type 1 (ARCA1) is an adult-onset slowly progressive cerebellar ataxia accompanied by dysarthria and other associated features[105]. It was first described and so far only reported in French-Canadian families most of them originating from defined regions of Quebec. A single locus on chromosome 6q25.1-q25.2 has been mapped and mutations in the *SYNE1* gene have been associated with the disease. Syne-1 is involved in anchoring specialized myonuclei underneath the neuromuscular junction[105] and plays a significant role in the development and maintenance of cerebellar functions. *SYNE1* is a large gene of 147 exons and this makes mutation screening by traditional methods expensive and complicated. Thus far direct sequencing of all the exons and flanking intronic sequences revealed a small number of missense and splice site mutations as well as a small scale deletion[105,106].

**Rundataxin-related ataxia:** A new form of pure recessive cerebellar ataxia accompanied by epilepsy and mental retardation named 'Salih ataxia' has been described in a large consanguineous Saudi Arabian family with three affected individuals[107]. Homozygosity mapping with SNP array (Gene Chip Human Mapping 10K 2.0 Xba Array, Affymetrix) and further linkage analysis with microsatellite markers, mapped the disease gene on chromosome 3q27.3-qter. Direct sequencing of the candidate genes enabled identification of the causative mutation in the *KIAA0226* gene. A single nucleotide deletion 2927delC [p.Ala943ValfsX146] frameshift mutation in this gene has thus far been identified. The encoded protein was named rundataxin based on its two conserved domains[107] and it is associated with vesicular trafficking and signaling pathways.

## NEXT-GENERATION SEQUENCING AND MOLECULAR DIAGNOSIS OF AUTOSOMAL RECESSIVE CEREBELLAR ATAXIAS

NGS technologies provide powerful tools for the identification of new genes. During the last 3 years, use of homozygosity mapping in combination with WES or targeted sequencing using the NGS platforms, enabled the identification of 5 new genes associated with rare types of ARCA in small families. WES has also been used as a screening tool thus enabling the rapid identification of mutations in known genes and demonstrating that it can be an efficient diagnostic tool.

### *Identification of new genes*

**Spinocerebellar ataxia autosomal recessive type 10:** A new form of pure recessive ataxia accompanied by downbeat nystagmus and lower motor neuropathy, first described in a Dutch consanguineous family and named spinocerebellar ataxia autosomal recessive type 10 (SCAR10), was the first recessive ataxia molecularly diagnosed with homozygosity mapping and NGS[108]. It is also known as ARCA3[2]. Homozygosity mapping with a 10K SNP array (Affymetrix) combined with linkage studies with short tandem repeat markers led to fine mapping of the disease gene to chromosome 3p21.32-p22.3. A large number of genes were included in the mapped region thus making identification of the causative mutation

with the traditional Sanger sequencing difficult. Use of Sanger sequencing to analyze 15 candidate genes failed to identify any mutation. Targeted NGS was subsequently performed that enabled identification of the causative mutation in the *ANO10* gene, thus demonstrating the power of this method towards the identification of new genes. A homozygous c.1529T > G [p.Leu510Arg] mutation was identified in the Dutch family. Additional mutations have been identified in patients originating from Serbia (a homozygous frameshift mutation c.1150_1151del [p.Leu384fs]) and France (compound heterozygous mutations c.1476 + 1G > T and c.1604del [p.Leu535X]) through direct analysis of the new gene by conventional Sanger sequencing[108]. More recently, a Japanese patient has been reported with a homozygous nonsense mutation (c.609C > G [p.Y203X]) identified by exome sequencing[19] indicating that this new method can be applied as a screening tool allowing the fast identification of mutations in known genes. The encoded protein *ANO10* is a member of the human anoctamin family and similar to the other proteins of this family it is a putative calcium-dependent chloride channel, thus representing a new pathway implicated in cerebellar ataxias. However, this function remains to be confirmed[108].

**Spinocerebellar ataxia autosomal recessive type 11:** A slowly progressive new type of ARCA accompanied by psychomotor retardation has been recently described in a small consanguineous Japanese family with two affected individuals and is known as spinocerebellar ataxia autosomal recessive type 11 (SCAR11). Homozygosity mapping using the Human SNP Array 6.0 (Affymetrix) combined with multipoint linkage analysis using extracted SNP array data revealed three candidate regions of homozygosity. WES was subsequently employed and data analysis focused on the regions of homozygosity. Sanger sequencing was used to further investigate filtered variants and the synaptotagmins 14 (SYT14) homozygous c.1451G > A [p.Gly484Asp] missense mutation has been associated with the disease. Further screening of all the coding regions of the entire gene in sporadic and familial ARCA cases failed to identify additional mutations. The encoded protein is Synaptotagmin XIV, a member of SYTs which are transmembrane proteins associated with exocytosis of secretory vesicles. Expression studies on SYT14 show that this protein plays a significant role in the cerebellum and support previous suggestions about the possible involvement of disrupted SYTs in neurodegeneration[12].

**Spinocerebellar ataxia autosomal recessive type 7:** Spinocerebellar ataxia autosomal recessive type 7 (SCAR7) was first described in a Dutch family in 2004 as a distinct ARCA type, based on its genetic locus, the onset of the disease and/or other clinical findings[109]. The disease gene has been mapped on chromosome 11p15 by genome-wide linkage analysis with microsatellite markers from the Marshfield genetic map. Identification of the causative mutation/gene was impossible for many years due to the large number (> 200) of genes within the candidate region. Recently, the use of WES enabled the association of the disease in the family with two *TPP1* compound heterozygous mutations (the splice site c.509-1G > C and the missense c.1397T > G [p.Val466Gly])[110]. These mutations were also detected in a sporadic SCAR7 patient. Mutations in the *TPP1* gene had already been associated with another disorder, the late infantile lipofuscinosis disease 2 (CLN2). Association of mutations in this gene with SCAR7 expands the phenotypes related to variants of the *TPP1* gene. Phenotype-genotype correlations proposed the hypothesis that CLN2 disease is due to variants causing the loss of enzyme activity, while SCAR7 is due to variants decreasing the enzyme activity. *TPP1* encodes the tripeptidyl-peptidase 1 enzyme, a member of the sedolisin family of serine proteases. This enzyme cleaves the N-terminal tripeptides from substrates and has a weak endopeptidase activity[110].

**Autosomal-recessive cerebellar ataxia with spasticity:** A distinct form of ataxia previously described in three consanguineous Tunisian families with 7 affected individuals has been recently molecularly characterized with the combined strategy of homozygosity mapping and WES thus providing further evidence about the potential of these methods[111]. Childhood or juvenile onset cerebellar ataxia was the main feature in all affected individuals of the families but later they developed pronounced limb spasticity. High density SNP genotyping was performed with the OmniExp-12, v1.0 DNA Analysis BeadChip (Illumina) and one large region of homozygosity on chromosome 9 was identified. The *APTX* gene mutated in AOA1 was included in the mapped region, so mutations in *APTX* were first excluded by Sanger sequencing. WES was then performed that revealed 2 causative mutations in the *GBA2* gene (the homozygous c.1018C > T [p.Arg340*] shared by two families and the homozygous c.2618G > A [p.Arg873His])[111]. Sanger sequencing confirmed segregation of the identified mutations with the disease in the families. Furthermore, WES was used to screen 21 molecularly undiagnosed Tunisian individuals with ataxia. Through this procedure an additional *GBA2* mutation (c.363C > A [p.Tyr121*]) was identified[111]. A second group associated 4 *GBA2* mutations with the spastic paraplegia type 46 (SPG46) phenotype[112].

*GBA2* encodes the non-lysosomal β-glucosidase 2 protein that was first recognized as a microsomal bile-acid β-glucosidase, while more recently it was reassigned to sphingolipid metabolism[113], a critical process for the nervous system[114]. The role of the enzyme in the central nervous system development was confirmed through the validation of the functional phenotype of some of the identified mutations causing HSP *in vivo* thus demonstrating the connection of sphingolipid metabolism and neurodegeneration[112]. HSPs are another heterogeneous group of neurodegenerative disorders having common features with ataxias and as a consequence, many times it is difficult for the clinician to decide whether the patient has hereditary ataxia with spasticity or HSP with cerebellar features. Identification of mutations in the same gene

causing these two diseases demonstrates that a common pathway may be involved in neurodegeneration.

**Spinocerebellar ataxia autosomal recessive type 13:** A new form of congenital cerebellar ataxia identified in Roma patients has recently been described and molecularly characterized[115]. This new form is known as spinocerebellar ataxia autosomal recessive type 13 (SCAR13). WES was used both for linkage analysis and mutation detection. A subgroup of SNP genotypes was first selected from the exome sequence data and used to perform genome wide linkage analysis, thus emphasizing an additional application of this method. Linkage analysis mapped the disease gene to a single locus on chromosome 6q24. Data analysis was focused on the linked interval and finally resulted to the identification of two homozygous mutations in the *GRM1* gene (c.2652_2654del [p.Asn885del] and c.2660+2T > G). Sanger sequencing was used to verify the presence of mutations and their co-segregation with the disease within the families while PCR-based restriction-fragment-length-polymorphism assays and pyrosequencing were used to evaluate the mutation frequencies among control individuals. Both mutations interfere with a critical alternative splicing gene region. *GRM1* encodes the metabotropic glutamate receptor mGluR1 which is implicated in the modulation of intracellular $Ca^{2+}$ levels and neuronal excitability. It is important for the development of the cerebellar cortex and the cerebellar and hippocampal synaptic plasticity, memory and learning[115].

### *Identification of mutations in known genes*

The combined strategy of homozygosity mapping and WES has been recently applied for the investigation of a consanguineous Turkish family and revealed a novel homozygous missense mutation (c.1366C > G [p.Leu456Val]) in the *C10orf2* gene, mutations in which have already been associated with infantile-onset spinocerebellar ataxia (IOSCA)[16]. This rare type of ataxia was previously reported only in Finland. The recent identification of this novel mutation shows that IOSCA is not restricted to Finland.

WES was applied in another recent study to investigate two affected non-consanguineous siblings with a multisystem neurological disorder of unknown genetic origin and revealed two novel heterozygous mutations in the known *SACS* gene. These patients had been previously investigated with conventional methods which failed to identify the associated mutations in contrast to this novel approach which rapidly led to molecular diagnosis. Both patients shared the compound heterozygous mutations c.2076delG [p.Thr692Thrfs*713] and c.3965_3966delAC [pGly1322Valfs*1343] in the *SACS* gene that have been associated with ARSACS. Another novel homozygous mutation in *SACS* (c.2439-2440delAT [p.Val815Glyfs*4]) has been identified in a Tunisian family with two affected individuals, by homozygosity mapping and WES[13]. A more recent study on a 4-year-old girl revealed an additional novel frameshift homozygous mutation in the same gene by WES[18].

WES also enabled the molecular diagnosis of the disease in an early onset spastic ataxia-neuropathy syndrome family. A homozygous missense mutation in the *AFG3L2* gene (c.1847G > A [p.Y616C]) has been identified[116]. Heterozygous mutations in the *AFG3L2* gene have previously been identified in autosomal dominant spinocerebellar ataxia type 28 patients[117,118]. The *AFG3L2* gene encodes a subunit of the m-AAA class of mitochondrial proteases which forms either a homo-oligomeric isoenzyme or a hetero-oligomeric complex with paraplegin, encoded by the *SPG7* gene[116]. Mutations in the *SPG7* gene have been associated with hereditary SPG7, characterized by adult-onset spasticity and weakness of the lower extremities[119]. In contrast to SCA28, this new syndrome associated with a recessive pattern of inheritance, is characterized by a much earlier onset and combines phenotypic features of SCA28 and *SPG7*[116].

## SYNOPSIS

The diagnosis of ARCA is becoming progressively more complex and challenging since clinical and genetic heterogeneity is expanding. Mutations in more than 20 genes have been associated with ARCA through the last decade, some of them identified in single families. There exists a significant number of patients remaining without a molecular diagnosis thus leading to the hypotheses that many other novel ARCA genes remain to be discovered in the future and that a different gene might be associated with the disease in each family mainly in populations with frequent inbreeding. The development of NGS technologies has already contributed significantly towards the molecular diagnosis of these rare diseases and appears very promising towards the diagnosis of additional cases in the future.

Sequencing of known candidate genes with conventional methods based on clinical findings has proved to be time-consuming and costly, except in the case of metabolic ataxias for which biochemical test results can precisely guide the molecular diagnosis. Routine investigation of some already known genes such as *SYNE1*, *ATM*, *SETX*, *POLG* and *SACS* is prohibitive, due to their significantly large coding regions.

Homozygosity mapping in consanguineous families has proved to be a powerful tool and may precede sequencing thus contributing to a more targeted analysis and facilitating the identification of novel ARCA genes. High density genome wide SNP genotyping with SNP arrays is the most rapid and effective method applied in the last years for homozygosity mapping. However, further analysis with more informative markers is often required in order to confirm or exclude some of the identified regions. Moreover, instead of using a SNP array, extracted SNP genotypes from the WES data may be used for genome wide linkage analysis. If a small number of genes exists within a mapped interval, then investigation by conventional sequencing may be efficient. Otherwise, if a large number of candidate genes exists, conventional

sequencing proves to be difficult, time consuming and expensive compared to the new methodologies.

WES enables the rapid sequencing of all coding regions of the genome and its usefulness has already been demonstrated in research and diagnostic applications. Conventional sequencing remains useful as a complimentary method for the investigation of the segregation pattern of candidate variants previously revealed by WES and verification of the causative mutations. Moreover, investigation of families for mutations in a known gene within a mapped region could be performed either by conventional sequencing or by WES depending on the size of the coding regions that need to be examined. For small coding regions, the first method may be more cost effective while for large genes, WES is less expensive since costs have significantly decreased over the last year[14].

Although the application of NGS technologies enables the identification of novel ARCA genes and the characterisation of novel mutations in known genes thus providing molecular diagnosis in many cases, there are some limitations due to their current reduced ability to sequence repetitive DNA expansions[14]. The most common form of ARCA is caused by the GAA repeat expansion in intron 1 of the *FXN* gene. Additional forms of ARCA with unknown genetic etiology may also be associated with a similar type of mutation and will not be identified with the NGS approach.

## CONCLUSION

Novel high-throughput sequencing technologies such as WES/WGS are ideal for simultaneously sequencing groups of candidate genes, whole-genome coding regions or the whole-genome and have brought a revolution in the molecular diagnosis of human genetic diseases. These technologies are capable of producing large amounts of data in one experiment and have been successfully used for the diagnosis of many rare and complex diseases including rare neurodegenerative diseases such as the ataxias. Their use for both research and diagnostic purposes has already contributed to the progress observed during the last years in the field of ataxias, including both the recessive and dominant forms. They are also very promising for the identification of many other new forms of ataxia in the near future since this group of disorders is very heterogeneous and a significant number of patients have already been excluded from many of the known genes. Determination of the genetic cause of each form is essential in order to initiate further investigation regarding the pathogenetic mechanisms and the potential therapies for these diseases.

## REFERENCES


1 **Palau F**, Espinós C. Autosomal recessive cerebellar ataxias. *Orphanet J Rare Dis* 2006; **1**: 47 [PMID: 17112370 DOI: 10.1186/1750-1172-1-47]

2 **Anheim M**, Tranchant C, Koenig M. The autosomal recessive cerebellar ataxias. *N Engl J Med* 2012; **366**: 636-646 [PMID: 22335741 DOI: 10.1056/NEJMra1006610]

3 **Anheim M**, Fleury M, Monga B, Laugel V, Chaigne D, Rodier G, Ginglinger E, Boulay C, Courtois S, Drouot N, Fritsch M, Delaunoy JP, Stoppa-Lyonnet D, Tranchant C, Koenig M. Epidemiological, clinical, paraclinical and molecular study of a cohort of 102 patients affected with autosomal recessive progressive cerebellar ataxia from Alsace, Eastern France: implications for clinical management. *Neurogenetics* 2010; **11**: 1-12 [PMID: 19440741 DOI: 10.1007/s10048-009-0196-y]

4 **Klockgether T**. Update on degenerative ataxias. *Curr Opin Neurol* 2011; **24**: 339-345 [PMID: 21734495 DOI: 10.1097/WCO.0b013e32834875ba]

5 **Espinós-Armero C**, González-Cabo P, Palau-Martínez F. [Autosomal recessive cerebellar ataxias. Their classification, genetic features and pathophysiology]. *Rev Neurol* 2005; **41**: 409-422 [PMID: 16193447]

6 **Dean G**, Chamberlain S, Middleton L. Friedreich's ataxia in Kathikas-Arodhes, Cyprus. *Lancet* 1988; **1**: 587 [PMID: 2894517]

7 **Zamba-Papanicolaou E**, Koutsou P, Daiou C, Gaglia E, Georghiou A, Christodoulou K. High frequency of Friedreich's ataxia carriers in the Paphos district of Cyprus. *Acta Myol* 2009; **28**: 24-26 [PMID: 19772192]

8 **Harding AE**. Classification of the hereditary ataxias and paraplegias. *Lancet* 1983; **1**: 1151-1155 [PMID: 6133167]

9 **Harding AE**. Clinical features and classification of inherited ataxias. *Adv Neurol* 1993; **61**: 1-14 [PMID: 8421960]

10 **Koenig M**. Rare forms of autosomal recessive neurodegenerative ataxia. *Semin Pediatr Neurol* 2003; **10**: 183-192 [PMID: 14653406 DOI: 10.1016/S1071-9091(03)00027-5]

11 **De Michele G**, Coppola G, Cocozza S, Filla A. A pathogenetic classification of hereditary ataxias: is the time ripe? *J Neurol* 2004; **251**: 913-922 [PMID: 15316795 DOI: 10.1007/s00415-004-0484-2]

12 **Doi H**, Yoshida K, Yasuda T, Fukuda M, Fukuda Y, Morita H, Ikeda S, Kato R, Tsurusaki Y, Miyake N, Saitsu H, Sakai H, Miyatake S, Shiina M, Nukina N, Koyano S, Tsuji S, Kuroiwa Y, Matsumoto N. Exome sequencing reveals a homozygous SYT14 mutation in adult-onset, autosomal-recessive spinocerebellar ataxia with psychomotor retardation. *Am J Hum Genet* 2011; **89**: 320-327 [PMID: 21835308 DOI: 10.1016/j.ajhg.2011.07.012]

13 **Hammer MB**, Eleuch-Fayache G, Gibbs JR, Arepalli SK, Chong SB, Sassi C, Bouhlal Y, Hentati F, Amouri R, Singleton AB. Exome sequencing: an efficient diagnostic tool for complex neurodegenerative disorders. *Eur J Neurol* 2013; **20**: 486-492 [PMID: 23043354 DOI: 10.1111/j.1468-1331.2012.03883.x]

14 **Sailer A**, Houlden H. Recent advances in the genetics of cerebellar ataxias. *Curr Neurol Neurosci Rep* 2012; **12**: 227-236 [PMID: 22527681 DOI: 10.1007/s11910-012-0267-6]

15 **H'mida-Ben Brahim D**, M'zahem A, Assoum M, Bouhlal Y, Fattori F, Anheim M, Ali-Pacha L, Ferrat F, Chaouch M, Lagier-Tourenne C, Drouot N, Thibaut C, Benhassine T, Sifi Y, Stoppa-Lyonnet D, N'Guyen K, Poujet J, Hamri A, Hentati F, Amouri R, Santorelli FM, Tazir M, Koenig M. Molecular diagnosis of known recessive ataxias by homozygosity mapping with SNP arrays. *J Neurol* 2011; **258**: 56-67 [PMID: 20798953 DOI: 10.1007/s00415-010-5682-5]

16 **Dündar H**, Ozgül RK, Yalnızoğlu D, Erdem S, Oğuz KK, Tuncel D, Temuçin CM, Dursun A. Identification of a novel Twinkle mutation in a family with infantile onset spinocerebellar ataxia by whole exome sequencing. *Pediatr Neurol* 2012; **46**: 172-177 [PMID: 22353293 DOI: 10.1016/j.pediatrneurol.2011.12.006]

17 **Pyle A**, Griffin H, Yu-Wai-Man P, Duff J, Eglon G, Pickering-Brown S, Santibanez-Korev M, Horvath R, Chinnery PF. Prominent sensorimotor neuropathy due to SACS mutations revealed by whole-exome sequencing. *Arch Neurol* 2012; **69**: 1351-1354 [PMID: 22751902 DOI: 10.1001/archneurol.2012.1472]

18 **Liew WK**, Ben-Omran T, Darras BT, Prabhu SP, De Vivo DC, Vatta M, Yang Y, Eng CM, Chung WK. Clinical application of whole-exome sequencing: a novel autosomal recessive spastic ataxia of Charlevoix-Saguenay sequence variation in a child with ataxia. *JAMA Neurol* 2013; **70**: 788-791 [PMID: 23699708 DOI: 10.1001/jamaneurol.2013.247]

19 **Maruyama H**, Morino H, Miyamoto R, Murakami N, Hamano T, Kawakami H. Exome sequencing reveals a novel ANO10 mutation in a Japanese patient with autosomal recessive spinocerebellar ataxia. *Clin Genet* 2013 Apr 4; Epub ahead of print [PMID: 23551081 DOI: 10.1111/cge.12140]

20 **Sailer A**, Scholz SW, Gibbs JR, Tucci A, Johnson JO, Wood NW, Plagnol V, Hummerich H, Ding J, Hernandez D, Hardy J, Federoff HJ, Traynor BJ, Singleton AB, Houlden H. Exome sequencing in an SCA14 family demonstrates its utility in diagnosing heterogeneous diseases. *Neurology* 2012; **79**: 127-131 [PMID: 22675081 DOI: 10.1212/WNL.0b013e31825f048e]

21 **Chen Z**, Wang JL, Tang BS, Sun ZF, Shi YT, Shen L, Lei LF, Wei XM, Xiao JJ, Hu ZM, Pan Q, Xia K, Zhang QY, Dai MZ, Liu Y, Ashizawa T, Jiang H. Using next-generation sequencing as a genetic diagnostic tool in rare autosomal recessive neurologic Mendelian disorders. *Neurobiol Aging* 2013; **34**: 2442.e11-7 [PMID: 23726790 DOI: 10.1016/j.neurobiolaging.2013.04.029]

22 **Cossée M**, Schmitt M, Campuzano V, Reutenauer L, Moutou C, Mandel JL, Koenig M. Evolution of the Friedreich's ataxia trinucleotide repeat expansion: founder effect and premutations. *Proc Natl Acad Sci USA* 1997; **94**: 7452-7457 [PMID: 9207112 DOI: 10.1073/pnas.94.14.7452]

23 **Delatycki MB**, Williamson R, Forrest SM. Friedreich ataxia: an overview. *J Med Genet* 2000; **37**: 1-8 [PMID: 10633128 DOI: 10.1136/jmg.37.1.1]

24 **Santos R**, Lefevre S, Sliwa D, Seguin A, Camadro JM, Lesuisse E. Friedreich ataxia: Molecular mechanisms, redox considerations, and therapeutic opportunities. *Antioxid Redox Sign* 2010; **13**: 651-690 [DOI: 10.1089/ars.2009.3015]

25 **Hanauer A**, Chery M, Fujita R, Driesel AJ, Gilgenkrantz S, Mandel JL. The Friedreich ataxia gene is assigned to chromosome 9q13-q21 by mapping of tightly linked markers and shows linkage disequilibrium with D9S15. *Am J Hum Genet* 1990; **46**: 133-137 [PMID: 2294745]

26 **Raimondi E**, Antonelli A, Driesel AJ, Pandolfo M. Regional localization by in situ hybridization of a human chromosome 9 marker tightly linked to the friedreich's ataxia locus. *Hum Genet* 1990; **85**: 125-126 [PMID: 1972693 DOI: 10.1007/BF00276338]

27 **Shaw J**, Lichter P, Driesel AJ, Williamson R, Chamberlain S. Regional localisation of the Friedreich ataxia locus to human chromosome 9q13----q21.1. *Cytogenet Cell Genet* 1990; **53**: 221-224 [PMID: 2209091 DOI: 10.1159/000132936]

28 **Campuzano V**, Montermini L, Moltò MD, Pianese L, Cossée M, Cavalcanti F, Monros E, Rodius F, Duclos F, Monticelli A, Zara F, Cañizares J, Koutnikova H, Bidichandani SI, Gellera C, Brice A, Trouillas P, De Michele G, Filla A, De Frutos R, Palau F, Patel PI, Di Donato S, Mandel JL, Cocozza S, Koenig M, Pandolfo M. Friedreich's ataxia: autosomal recessive disease caused by an intronic GAA triplet repeat expansion. *Science* 1996; **271**: 1423-1427 [PMID: 8596916 DOI: 10.1126/science.271.5254.1423]

29 **Cossée M**, Dürr A, Schmitt M, Dahl N, Trouillas P, Allinson P, Kostrzewa M, Nivelon-Chevallier A, Gustavson KH, Kohlschütter A, Müller U, Mandel JL, Brice A, Koenig M, Cavalcanti F, Tammaro A, De Michele G, Filla A, Cocozza S, Labuda M, Montermini L, Poirier J, Pandolfo M. Friedreich's ataxia: point mutations and clinical presentation of compound heterozygotes. *Ann Neurol* 1999; **45**: 200-206 [PMID: 9989622 DOI: 10.1002/1531-8249(199902)45: 2<200: : AID-ANA10>3.0.CO;2-U]

30 **Pandolfo M**. Friedreich ataxia. *Arch Neurol* 2008; **65**: 1296-1303 [PMID: 18852343 DOI: 10.1001/archneur.65.10.1296]

31 **Filla A**, De Michele G, Cavalcanti F, Pianese L, Monticelli A, Campanella G, Cocozza S. The relationship between trinucleotide (GAA) repeat length and clinical features in Friedreich ataxia. *Am J Hum Genet* 1996; **59**: 554-560 [PMID: 8751856]

32 **Ciotti P**, Di Maria E, Bellone E, Ajmar F, Mandich P. Triplet repeat primed PCR (TP PCR) in molecular diagnostic testing for Friedreich ataxia. *J Mol Diagn* 2004; **6**: 285-289 [PMID: 15507666 DOI: 10.1016/S1525-1578(10)60523-5]

33 **Warner JP**, Barron LH, Goudie D, Kelly K, Dow D, Fitzpatrick DR, Brock DJ. A general method for the detection of large CAG repeat expansions by fluorescent PCR. *J Med Genet* 1996; **33**: 1022-1026 [PMID: 9004136 DOI: 10.1136/jmg.33.12.1022]

34 **Hakonen AH**, Heiskanen S, Juvonen V, Lappalainen I, Luoma PT, Rantamaki M, Goethem GV, Lofgren A, Hackman P, Paetau A, Kaakkola S, Majamaa K, Varilo T, Udd B, Kaariainen H, Bindoff LA, Suomalainen A. Mitochondrial DNA polymerase W748S mutation: a common cause of autosomal recessive ataxia with ancient European origin. *Am J Hum Genet* 2005; **77**: 430-441 [PMID: 16080118 DOI: 10.1086/444548]

35 **Van Goethem G**, Luoma P, Rantamäki M, Al Memar A, Kaakkola S, Hackman P, Krahe R, Löfgren A, Martin JJ, De Jonghe P, Suomalainen A, Udd B, Van Broeckhoven C. POLG mutations in neurodegenerative disorders with ataxia but no muscle involvement. *Neurology* 2004; **63**: 1251-1257 [PMID: 15477547 DOI: 10.1212/01.WNL.0000140494.58732.83]

36 **Winterthun S**, Ferrari G, He L, Taylor RW, Zeviani M, Turnbull DM, Engelsen BA, Moen G, Bindoff LA. Autosomal recessive mitochondrial ataxic syndrome due to mitochondrial polymerase gamma mutations. *Neurology* 2005; **64**: 1204-1208 [PMID: 15824347 DOI: 10.1212/01.WNL.0000156516.77696.5A]

37 **Nikali K**, Suomalainen A, Saharinen J, Kuokkanen M, Spelbrink JN, Lönnqvist T, Peltonen L. Infantile onset spinocerebellar ataxia is caused by recessive mutations in mitochondrial proteins Twinkle and Twinky. *Hum Mol Genet* 2005; **14**: 2981-2990 [PMID: 16135556 DOI: 10.1093/hmg/ddi328]

38 **Richter A**, Rioux JD, Bouchard JP, Mercier J, Mathieu J, Ge B, Poirier J, Julien D, Gyapay G, Weissenbach J, Hudson TJ, Melançon SB, Morgan K. Location score and haplotype analyses of the locus for autosomal recessive spastic ataxia of Charlevoix-Saguenay, in chromosome region 13q11. *Am J Hum Genet* 1999; **64**: 768-775 [PMID: 10053011 DOI: 10.1086/302274]

39 **Mercier J**, Prévost C, Engert JC, Bouchard JP, Mathieu J, Richter A. Rapid detection of the sacsin mutations causing autosomal recessive spastic ataxia of Charlevoix-Saguenay. *Genet Test* 2001; **5**: 255-259 [PMID: 11788093 DOI: 10.1089/109065701527423 26]

40 **Engert JC**, Bérubé P, Mercier J, Doré C, Lepage P, Ge B, Bouchard JP, Mathieu J, Melançon SB, Schalling M, Lander ES, Morgan K, Hudson TJ, Richter A. ARSACS, a spastic ataxia common in northeastern Québec, is caused by mutations in a new gene encoding an 11.5-kb ORF. *Nat Genet* 2000; **24**: 120-125 [PMID: 10655055 DOI: 10.1038/72769]

41 **Synofzik M**, Soehn AS, Gburek-Augustat J, Schicks J, Karle KN, Schüle R, Haack TB, Schöning M, Biskup S, Rudnik-Schöneborn S, Senderek J, Hoffmann KT, MacLeod P, Schwarz J, Bender B, Krüger S, Kreuz F, Bauer P, Schöls L. Autosomal recessive spastic ataxia of Charlevoix Saguenay (ARSACS): expanding the genetic, clinical and imaging spectrum. *Orphanet J Rare Dis* 2013; **8**: 41 [PMID: 23497566 DOI: 10.1186/1750-1172-8-41]

42 **Ouyang Y**, Takiyama Y, Sakoe K, Shimazaki H, Ogawa T, Nagano S, Yamamoto Y, Nakano I. Sacsin-related ataxia (ARSACS): expanding the genotype upstream from the gigantic exon. *Neurology* 2006; **66**: 1103-1104 [PMID: 16606928 DOI: 10.1212/01.wnl.0000204300.94261.ea]

43 **Vermeer S**, Meijer RP, Pijl BJ, Timmermans J, Cruysberg

JR, Bos MM, Schelhaas HJ, van de Warrenburg BP, Knoers NV, Scheffer H, Kremer B. ARSACS in the Dutch population: a frequent cause of early-onset cerebellar ataxia. *Neurogenetics* 2008; **9**: 207-214 [PMID: 18465152 DOI: 10.1007/s10048-008-0131-7]

44 **Vermeer S**, van de Warrenburg BP, Kamsteeg EJ. ARSACS. 2003 dec 9 [updated 2012 oct 11]. In: Pagon RA, Adam MP, Bird TD, Dolan CR, Fong CT and Stephens K Genereviews [internet]. Seattle (WA). University of Washington, Seattle; 1993-2013

45 **Lagier-Tourenne C**, Tranebaerg L, Chaigne D, Gribaa M, Dollfus H, Silvestri G, Bétard C, Warter JM, Koenig M. Homozygosity mapping of Marinesco-Sjögren syndrome to 5q31. *Eur J Hum Genet* 2003; **11**: 770-778 [PMID: 14512967 DOI: 10.1038/sj.ejhg.5201068]

46 **Anttonen AK**, Mahjneh I, Hämäläinen RH, Lagier-Tourenne C, Kopra O, Waris L, Anttonen M, Joensuu T, Kalimo H, Paetau A, Tranebjaerg L, Chaigne D, Koenig M, Eeg-Olofsson O, Udd B, Somer M, Somer H, Lehesjoki AE. The gene disrupted in Marinesco-Sjögren syndrome encodes SIL1, an HSPA5 cochaperone. *Nat Genet* 2005; **37**: 1309-1311 [PMID: 16282978 DOI: 10.1038/ng1677]

47 **Senderek J**, Krieger M, Stendel C, Bergmann C, Moser M, Breitbach-Faller N, Rudnik-Schöneborn S, Blaschek A, Wolf NI, Harting I, North K, Smith J, Muntoni F, Brockington M, Quijano-Roy S, Renault F, Herrmann R, Hendershot LM, Schröder JM, Lochmüller H, Topaloglu H, Voit T, Weis J, Ebinger F, Zerres K. Mutations in SIL1 cause Marinesco-Sjögren syndrome, a cerebellar ataxia with cataract and myopathy. *Nat Genet* 2005; **37**: 1312-1314 [PMID: 16282977]

48 **Anttonen AK**, Siintola E, Tranebjaerg L, Iwata NK, Bijlsma EK, Meguro H, Ichikawa Y, Goto J, Kopra O, Lehesjoki AE. Novel SIL1 mutations and exclusion of functional candidate genes in Marinesco-Sjögren syndrome. *Eur J Hum Genet* 2008; **16**: 961-969 [PMID: 18285827 DOI: 10.1038/ejhg.2008.22]

49 **Lagier-Tourenne C**, Tazir M, López LC, Quinzii CM, Assoum M, Drouot N, Busso C, Makri S, Ali-Pacha L, Benhassine T, Anheim M, Lynch DR, Thibault C, Plewniak F, Bianchetti L, Tranchant C, Poch O, DiMauro S, Mandel JL, Barros MH, Hirano M, Koenig M. ADCK3, an ancestral kinase, is mutated in a form of recessive ataxia associated with coenzyme Q10 deficiency. *Am J Hum Genet* 2008; **82**: 661-672 [PMID: 18319074 DOI: 10.1016/j.ajhg.2007.12.024]

50 **Horvath R**, Czermin B, Gulati S, Demuth S, Houge G, Pyle A, Dineiger C, Blakely EL, Hassani A, Foley C, Brodhun M, Storm K, Kirschner J, Gorman GS, Lochmüller H, Holinski-Feder E, Taylor RW, Chinnery PF. Adult-onset cerebellar ataxia due to mutations in CABC1/ADCK3. *J Neurol Neurosurg Psychiatry* 2012; **83**: 174-178 [PMID: 22036850 DOI: 10.1136/jnnp-2011-301258]

51 **Mollet J**, Delahodde A, Serre V, Chretien D, Schlemmer D, Lombes A, Boddaert N, Desguerre I, de Lonlay P, de Baulny HO, Munnich A, Rötig A. CABC1 gene mutations cause ubiquinone deficiency with cerebellar ataxia and seizures. *Am J Hum Genet* 2008; **82**: 623-630 [PMID: 18319072 DOI: 10.1016/j.ajhg.2007.12.022]

52 **Anheim M**, Monga B, Fleury M, Charles P, Barbot C, Salih M, Delaunoy JP, Fritsch M, Arning L, Synofzik M, Schöls L, Sequeiros J, Goizet C, Marelli C, Le Ber I, Koht J, Gazulla J, De Bleecker J, Mukhtar M, Drouot N, Ali-Pacha L, Benhassine T, Chbicheb M, M'Zahem A, Hamri A, Chabrol B, Pouget J, Murphy R, Watanabe M, Coutinho P, Tazir M, Durr A, Brice A, Tranchant C, Koenig M. Ataxia with oculomotor apraxia type 2: clinical, biological and genotype/phenotype correlation study of a cohort of 90 patients. *Brain* 2009; **132**: 2688-2698 [PMID: 19696032 DOI: 10.1093/brain/awp211]

53 **Suraweera A**, Lim Y, Woods R, Birrell GW, Nasim T, Becherel OJ, Lavin MF. Functional role for senataxin, defective in ataxia oculomotor apraxia type 2, in transcriptional regulation. *Hum Mol Genet* 2009; **18**: 3384-3396 [PMID: 19515850 DOI: 10.1093/hmg/ddp278]

54 **Al Tassan N**, Khalil D, Shinwari J, Al Sharif L, Bavi P, Abduljaleel Z, Abu Dhaim N, Magrashi A, Bobis S, Ahmed H, Alahmed S, Bohlega S. A missense mutation in PIK3R5 gene in a family with ataxia and oculomotor apraxia. *Hum Mutat* 2012; **33**: 351-354 [PMID: 22065524 DOI: 10.1002/humu.21650]

55 **Gatti RA**, Berkel I, Boder E, Braedt G, Charmley P, Concannon P, Ersoy F, Foroud T, Jaspers NG, Lange K, *et al*. Localization of an ataxia-telangiectasia gene to chromosome 11q22-23. *Nature* 1988; **336**: 577-580 [PMID: 3200306 DOI: 10.1038/336577a0]

56 **Gatti RA**, Lange E, Rotman G, Chen X, Uhrhammer N, Liang T, Chiplunkar S, Yang L, Udar N, Dandekar S, Sheikhavandi S, Wang Z, Yang HM, Polikow J, Elashoff M, Teletar M, Sanal O, Chessa L, McConville C, Taylor M, Shiloh Y, Porras O, Borresen AL, Wegner RD, Curry C, Gerken S, Lange K, Concannon P. Genetic haplotyping of ataxia-telangiectasia families localizes the major gene to an approximately 850 kb region on chromosome 11q23.1. *Int J Radiat Biol* 1994; **66**: S57-S62 [PMID: 7836853 DOI: 10.1080/09553009414551861]

57 **Buzin CH**, Gatti RA, Nguyen VQ, Wen CY, Mitui M, Sanal O, Chen JS, Nozari G, Mengos A, Li X, Fujimura F, Sommer SS. Comprehensive scanning of the ATM gene with DOVAM-S. *Hum Mutat* 2003; **21**: 123-131 [PMID: 12552559 DOI: 10.1002/humu.10158]

58 **Taylor AM**, Groom A, Byrd PJ. Ataxia-telangiectasia-like disorder (ATLD)-its clinical presentation and molecular basis. *DNA Repair (Amst)* 2004; **3**: 1219-1225 [PMID: 15279810 DOI: 10.1016/j.dnarep.2004.04.009]

59 **Stewart GS**, Maser RS, Stankovic T, Bressan DA, Kaplan MI, Jaspers NG, Raams A, Byrd PJ, Petrini JH, Taylor AM. The DNA double-strand break repair gene hMRE11 is mutated in individuals with an ataxia-telangiectasia-like disorder. *Cell* 1999; **99**: 577-587 [PMID: 10612394 DOI: 10.1016/S0092-8674(00)81547-0]

60 **Delia D**, Piane M, Buscemi G, Savio C, Palmeri S, Lulli P, Carlessi L, Fontanella E, Chessa L. MRE11 mutations and impaired ATM-dependent responses in an Italian family with ataxia-telangiectasia-like disorder. *Hum Mol Genet* 2004; **13**: 2155-2163 [PMID: 15269180 DOI: 10.1093/hmg/ddh221]

61 **Fernet M**, Gribaa M, Salih MA, Seidahmed MZ, Hall J, Koenig M. Identification and functional consequences of a novel MRE11 mutation affecting 10 Saudi Arabian patients with the ataxia telangiectasia-like disorder. *Hum Mol Genet* 2005; **14**: 307-318 [PMID: 15574463 DOI: 10.1093/hmg/ddi027]

62 **Moreira MC**, Barbot C, Tachi N, Kozuka N, Mendonça P, Barros J, Coutinho P, Sequeiros J, Koenig M. Homozygosity mapping of Portuguese and Japanese forms of ataxia-oculomotor apraxia to 9p13, and evidence for genetic heterogeneity. *Am J Hum Genet* 2001; **68**: 501-508 [PMID: 11170899]

63 **Date H**, Onodera O, Tanaka H, Iwabuchi K, Uekawa K, Igarashi S, Koike R, Hiroi T, Yuasa T, Awaya Y, Sakai T, Takahashi T, Nagatomo H, Sekijima Y, Kawachi I, Takiyama Y, Nishizawa M, Fukuhara N, Saito K, Sugano S, Tsuji S. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia is caused by mutations in a new HIT superfamily gene. *Nat Genet* 2001; **29**: 184-188 [PMID: 11586299 DOI: 10.1038/ng1001-184]

64 **Gueven N**, Becherel OJ, Kijas AW, Chen P, Howe O, Rudolph JH, Gatti R, Date H, Onodera O, Taucher-Scholz G, Lavin MF. Aprataxin, a novel protein that protects against genotoxic stress. *Hum Mol Genet* 2004; **13**: 1081-1093 [PMID: 15044383 DOI: 10.1093/hmg/ddh122]

65 **Sykora P**, Croteau DL, Bohr VA, Wilson DM. Aprataxin localizes to mitochondria and preserves mitochondrial function. *Proc Natl Acad Sci USA* 2011; **108**: 7437-7442 [PMID: 21502511 DOI: 10.1073/pnas.1100084108]

66 **Amouri R**, Moreira MC, Zouari M, El Euch G, Barhoumi C, Kefi M, Belal S, Koenig M, Hentati F. Aprataxin gene mutations in Tunisian families. *Neurology* 2004; **63**: 928-929 [PMID:

15365154 DOI: 10.1212/01.WNL.0000137044.06573.46]
67 **Castellotti B**, Mariotti C, Rimoldi M, Fancellu R, Plumari M, Caimi S, Uziel G, Nardocci N, Moroni I, Zorzi G, Pareyson D, Di Bella D, Di Donato S, Taroni F, Gellera C. Ataxia with oculomotor apraxia type1 (AOA1): novel and recurrent aprataxin mutations, coenzyme Q10 analyses, and clinical findings in Italian patients. *Neurogenetics* 2011; **12**: 193-201 [PMID: 21465257 DOI: 10.1007/s10048-011-0281-x]
68 **Nouri N**, Nouri N, Aryani O, Kamalidehghan B, Sedghi M, Houshmand M. A novel mutation in the aprataxin (APTX) gene in an Iranian individual suffering early-onset ataxia with oculomotor apraxia type 1(AOA1) disease. *Iran Biomed J* 2012; **16**: 223-225 [PMID: 23183622]
69 **Rudenskaia GE**, Kurkina MV, Zakharova EIu. [Ataxia with oculomotor apraxia: clinical-genetic characteristics and DNA-diagnostic]. *Zh Nevrol Psikhiatr Im S S Korsakova* 2012; **112**: 58-63 [PMID: 23250602]
70 **Yoon G**, Westmacott R, MacMillan L, Quercia N, Koutsou P, Georghiou A, Christodoulou K, Banwell B. Complete deletion of the aprataxin gene: ataxia with oculomotor apraxia type 1 with severe phenotype and cognitive deficit. *J Neurol Neurosurg Psychiatry* 2008; **79**: 234-236 [PMID: 18202221 DOI: 10.1136/jnnp.2007.127233]
71 **Moreira MC**, Klur S, Watanabe M, Németh AH, Le Ber I, Moniz JC, Tranchant C, Aubourg P, Tazir M, Schöls L, Pandolfo M, Schulz JB, Pouget J, Calvas P, Shizuka-Ikeda M, Shoji M, Tanaka M, Izatt L, Shaw CE, M'Zahem A, Dunne E, Bomont P, Benhassine T, Bouslam N, Stevanin G, Brice A, Guimarães J, Mendonça P, Barbot C, Coutinho P, Sequeiros J, Dürr A, Warter JM, Koenig M. Senataxin, the ortholog of a yeast RNA helicase, is mutant in ataxia-ocular apraxia 2. *Nat Genet* 2004; **36**: 225-227 [PMID: 14770181 DOI: 10.1038/ng1303]
72 **Hammer MB**, El Euch-Fayache G, Nehdi H, Saidi D, Nasri A, Nabli F, Bouhlal Y, Maamouri-Hicheri W, Hentati F, Amouri R. Clinical and molecular findings of ataxia with oculomotor apraxia type 2 (AOA2) in 5 Tunisian families. *Diagn Mol Pathol* 2012; **21**: 241-245 [PMID: 23111195 DOI: 10.1097/PDM.0b013e318257ad9a]
73 **Nicolaou P**, Georghiou A, Votsi C, Middleton LT, Zamba-Papanicolaou E, Christodoulou K. A novel c.5308_5311delGAGA mutation in Senataxin in a Cypriot family with an autosomal recessive cerebellar ataxia. *BMC Med Genet* 2008; **9**: 28 [PMID: 18405395 DOI: 10.1186/1471-2350-9-28]
74 **Bernard V**, Minnerop M, Bürk K, Kreuz F, Gillessen-Kaesbach G, Zühlke C. Exon deletions and intragenic insertions are not rare in ataxia with oculomotor apraxia 2. *BMC Med Genet* 2009; **10**: 87 [PMID: 2749023 DOI: 10.1186/1471-2350-10-87]
75 **Takashima H**, Boerkoel CF, John J, Saifi GM, Salih MA, Armstrong D, Mao Y, Quiocho FA, Roa BB, Nakagawa M, Stockton DW, Lupski JR. Mutation of TDP1, encoding a topoisomerase I-dependent DNA damage repair enzyme, in spinocerebellar ataxia with axonal neuropathy. *Nat Genet* 2002; **32**: 267-272 [PMID: 12244316 DOI: 10.1038/ng987]
76 **Hirano R**, Interthal H, Huang C, Nakamura T, Deguchi K, Choi K, Bhattacharjee MB, Arimura K, Umehara F, Izumo S, Northrop JL, Salih MA, Inoue K, Armstrong DL, Champoux JJ, Takashima H, Boerkoel CF. Spinocerebellar ataxia with axonal neuropathy: consequence of a Tdp1 recessive neomorphic mutation? *EMBO J* 2007; **26**: 4732-4743 [PMID: 17948061 DOI: 10.1038/sj.emboj.7601885]
77 **Bielas SL**, Silhavy JL, Brancati F, Kisseleva MV, Al-Gazali L, Sztriha L, Bayoumi RA, Zaki MS, Abdel-Aleem A, Rosti RO, Kayserili H, Swistun D, Scott LC, Bertini E, Boltshauser E, Fazzi E, Travaglini L, Field SJ, Gayral S, Jacoby M, Schurmans S, Dallapiccola B, Majerus PW, Valente EM, Gleeson JG. Mutations in INPP5E, encoding inositol polyphosphate-5-phosphatase E, link phosphatidyl inositol signaling to the ciliopathies. *Nat Genet* 2009; **41**: 1032-1036 [PMID: 19668216 DOI: 10.1038/ng.423]
78 **Edvardson S**, Shaag A, Zenvirt S, Erlich Y, Hannon GJ, Shanske AL, Gomori JM, Ekstein J, Elpeleg O. Joubert syndrome 2 (JBTS2) in Ashkenazi Jews is associated with a TMEM216 mutation. *Am J Hum Genet* 2010; **86**: 93-97 [PMID: 20036350 DOI: 10.1016/j.ajhg.2009.12.007]
79 **Ferland RJ**, Eyaid W, Collura RV, Tully LD, Hill RS, Al-Nouri D, Al-Rumayyan A, Topcu M, Gascon G, Bodell A, Shugart YY, Ruvolo M, Walsh CA. Abnormal cerebellar development and axonal decussation due to mutations in AHI1 in Joubert syndrome. *Nat Genet* 2004; **36**: 1008-1013 [PMID: 15322546 DOI: 10.1038/ng1419]
80 **Parisi MA**, Bennett CL, Eckert ML, Dobyns WB, Gleeson JG, Shaw DW, McDonald R, Eddy A, Chance PF, Glass IA. The NPHP1 gene deletion associated with juvenile nephronophthisis is present in a subset of individuals with Joubert syndrome. *Am J Hum Genet* 2004; **75**: 82-91 [PMID: 15138899 DOI: 10.1086/421846]
81 **Sayer JA**, Otto EA, O'Toole JF, Nurnberg G, Kennedy MA, Becker C, Hennies HC, Helou J, Attanasio M, Fausett BV, Utsch B, Khanna H, Liu Y, Drummond I, Kawakami I, Kusakabe T, Tsuda M, Ma L, Lee H, Larson RG, Allen SJ, Wilkinson CJ, Nigg EA, Shou C, Lillo C, Williams DS, Hoppe B, Kemper MJ, Neuhaus T, Parisi MA, Glass IA, Petry M, Kispert A, Gloy J, Ganner A, Walz G, Zhu X, Goldman D, Nurnberg P, Swaroop A, Leroux MR, Hildebrandt F. The centrosomal protein nephrocystin-6 is mutated in Joubert syndrome and activates transcription factor ATF4. *Nat Genet* 2006; **38**: 674-681 [PMID: 16682973 DOI: 10.1038/ng1786]
82 **Baala L**, Romano S, Khaddour R, Saunier S, Smith UM, Audollent S, Ozilou C, Faivre L, Laurent N, Foliguet B, Munnich A, Lyonnet S, Salomon R, Encha-Razavi F, Gubler MC, Boddaert N, de Lonlay P, Johnson CA, Vekemans M, Antignac C, Attie-Bitach T. The Meckel-Gruber syndrome gene, MKS3, is mutated in Joubert syndrome. *Am J Hum Genet* 2007; **80**: 186-194 [PMID: 17160906 DOI: 10.1086/510499]
83 **Delous M**, Baala L, Salomon R, Laclef C, Vierkotten J, Tory K, Golzio C, Lacoste T, Besse L, Ozilou C, Moutkine I, Hellman NE, Anselme I, Silbermann F, Vesque C, Gerhardt C, Rattenberry E, Wolf MT, Gubler MC, Martinovic J, Encha-Razavi F, Boddaert N, Gonzales M, Macher MA, Nivet H, Champion G, Berthélémé JP, Niaudet P, McDonald F, Hildebrandt F, Johnson CA, Vekemans M, Antignac C, Rüther U, Schneider-Maunoury S, Attié-Bitach T, Saunier S. The ciliary gene RPGRIP1L is mutated in cerebello-oculo-renal syndrome (Joubert syndrome type B) and Meckel syndrome. *Nat Genet* 2007; **39**: 875-881 [PMID: 17558409 DOI: 10.1038/ng2039]
84 **Bomar JM**, Benke PJ, Slattery EL, Puttagunta R, Taylor LP, Seong E, Nystuen A, Chen W, Albin RL, Patel PD, Kittles RA, Sheffield VC, Burmeister M. Mutations in a novel gene encoding a CRAL-TRIO domain cause human Cayman ataxia and ataxia/dystonia in the jittery mouse. *Nat Genet* 2003; **35**: 264-269 [PMID: 14556008 DOI: 10.1038/ng1255]
85 **Gieselmann V**, Fluharty AL, Tønnesen T, Von Figura K. Mutations in the arylsulfatase A pseudodeficiency allele causing metachromatic leukodystrophy. *Am J Hum Genet* 1991; **49**: 407-413 [PMID: 1678251]
86 **Polten A**, Fluharty AL, Fluharty CB, Kappler J, von Figura K, Gieselmann V. Molecular basis of different forms of metachromatic leukodystrophy. *N Engl J Med* 1991; **324**: 18-22 [PMID: 1670590 DOI: 10.1056/NEJM199101033240104]
87 **Vanier MT**, Millat G. Niemann-Pick disease type C. *Clin Genet* 2003; **64**: 269-281 [PMID: 12974729 DOI: 10.1034/j.1399-0004.2003.00147.x]
88 **Yoshida K**, Oshima A, Shimmoto M, Fukuhara Y, Sakuraba H, Yanagisawa N, Suzuki Y. Human beta-galactosidase gene mutations in GM1-gangliosidosis: a common mutation among Japanese adult/chronic cases. *Am J Hum Genet* 1991; **49**: 435-442 [PMID: 1907800]
89 **Nishimoto J**, Nanba E, Inui K, Okada S, Suzuki K. GM1-gangliosidosis (genetic beta-galactosidase deficiency): identification of four mutations in different clinical phenotypes among Japanese patients. *Am J Hum Genet* 1991; **49**: 566-574

[PMID: 1909089]
90 **Myerowitz R**, Costigan FC. The major defect in Ashkenazi Jews with Tay-Sachs disease is an insertion in the gene for the alpha-chain of beta-hexosaminidase. *J Biol Chem* 1988; **263**: 18587-18589 [PMID: 2848800]
91 **Myerowitz R**. Tay-Sachs disease-causing mutations and neutral polymorphisms in the Hex A gene. *Hum Mutat* 1997; **9**: 195-208 [PMID: 9090523 DOI: 10.1002/(SICI)1098-1004(1997)9: 3<195: : AID-HUMU1>3.0.CO; 2-7]
92 **Bull PC**, Thomas GR, Rommens JM, Forbes JR, Cox DW. The Wilson disease gene is a putative copper transporting P-type ATPase similar to the Menkes gene. *Nat Genet* 1993; **5**: 327-337 [PMID: 8298639 DOI: 10.1038/ng1293-327]
93 **Tanzi RE**, Petrukhin K, Chernov I, Pellequer JL, Wasco W, Ross B, Romano DM, Parano E, Pavone L, Brzustowicz LM. The Wilson disease gene is a copper transporting ATPase with homology to the Menkes disease gene. *Nat Genet* 1993; **5**: 344-350 [PMID: 8298641 DOI: 10.1038/ng1293-344]
94 **Thomas GR**, Forbes JR, Roberts EA, Walshe JM, Cox DW. The Wilson disease gene: spectrum of mutations and their consequences. *Nat Genet* 1995; **9**: 210-217 [PMID: 7626145 DOI: 10.1038/ng0295-210]
95 **Harris ZL**, Takahashi Y, Miyajima H, Serizawa M, MacGillivray RT, Gitlin JD. Aceruloplasminemia: molecular characterization of this disorder of iron metabolism. *Proc Natl Acad Sci USA* 1995; **92**: 2539-2543 [PMID: 7708681 DOI: 10.1073/pnas.92.7.2539]
96 **Rampoldi L**, Dobson-Stone C, Rubio JP, Danek A, Chalmers RM, Wood NW, Verellen C, Ferrer X, Malandrini A, Fabrizi GM, Brown R, Vance J, Pericak-Vance M, Rudolf G, Carrè S, Alonso E, Manfredi M, Németh AH, Monaco AP. A conserved sorting-associated protein is mutant in chorea-acanthocytosis. *Nat Genet* 2001; **28**: 119-120 [PMID: 11381253 DOI: 10.1038/88821]
97 **Ouahchi K**, Arita M, Kayden H, Hentati F, Ben Hamida M, Sokol R, Arai H, Inoue K, Mandel JL, Koenig M. Ataxia with isolated vitamin E deficiency is caused by mutations in the alpha-tocopherol transfer protein. *Nat Genet* 1995; **9**: 141-145 [PMID: 7719340 DOI: 10.1038/ng0295-141]
98 **Zamel R**, Khan R, Pollex RL, Hegele RA. Abetalipoproteinemia: two case reports and literature review. *Orphanet J Rare Dis* 2008; **3**: 19 [PMID: 18611256 DOI: 10.1186/1750-1172-3-19]
99 **Sharp D**, Blinderman L, Combs KA, Kienzle B, Ricci B, Wager-Smith K, Gil CM, Turck CW, Bouma ME, Rader DJ. Cloning and gene defects in microsomal triglyceride transfer protein associated with abetalipoproteinaemia. *Nature* 1993; **365**: 65-69 [PMID: 8361539 DOI: 10.1038/365065a0]
100 **Cali JJ**, Hsieh CL, Francke U, Russell DW. Mutations in the bile acid biosynthetic enzyme sterol 27-hydroxylase underlie cerebrotendinous xanthomatosis. *J Biol Chem* 1991; **266**: 7779-7783 [PMID: 2019602]
101 **Dubrac S**, Lear SR, Ananthanarayanan M, Balasubramaniyan N, Bollineni J, Shefer S, Hyogo H, Cohen DE, Blanche PJ, Krauss RM, Batta AK, Salen G, Suchy FJ, Maeda N, Erickson SK. Role of CYP27A in cholesterol and bile acid metabolism. *J Lipid Res* 2005; **46**: 76-85 [PMID: 15520450 DOI: 10.1194/jlr.M400219-JLR200]
102 **Jansen GA**, Ofman R, Ferdinandusse S, Ijlst L, Muijsers AO, Skjeldal OH, Stokke O, Jakobs C, Besley GT, Wraith JE, Wanders RJ. Refsum disease is caused by mutations in the phytanoyl-CoA hydroxylase gene. *Nat Genet* 1997; **17**: 190-193 [PMID: 9326940 DOI: 10.1038/ng1097-190]
103 **Mihalik SJ**, Morrell JC, Kim D, Sacksteder KA, Watkins PA, Gould SJ. Identification of PAHX, a Refsum disease gene. *Nat Genet* 1997; **17**: 185-189 [PMID: 9326939 DOI: 10.1038/ng1097-185]
104 **van den Brink DM**, Brites P, Haasjes J, Wierzbicki AS, Mitchell J, Lambert-Hamill M, de Belleroche J, Jansen GA, Waterham HR, Wanders RJ. Identification of PEX7 as the second gene involved in Refsum disease. *Am J Hum Genet* 2003; **72**: 471-477 [PMID: 12522768 DOI: 10.1086/346093]
105 **Gros-Louis F**, Dupré N, Dion P, Fox MA, Laurent S, Verreault S, Sanes JR, Bouchard JP, Rouleau GA. Mutations in SYNE1 lead to a newly discovered form of autosomal recessive cerebellar ataxia. *Nat Genet* 2007; **39**: 80-85 [PMID: 17159980 DOI: 10.1038/ng1927]
106 **Dupré N**, Gros-Louis F, Chrestian N, Verreault S, Brunet D, de Verteuil D, Brais B, Bouchard JP, Rouleau GA. Clinical and genetic study of autosomal recessive cerebellar ataxia type 1. *Ann Neurol* 2007; **62**: 93-98 [PMID: 17503513 DOI: 10.1002/ana.21143]
107 **Assoum M**, Salih MA, Drouot N, H'Mida-Ben Brahim D, Lagier-Tourenne C, AlDrees A, Elmalik SA, Ahmed TS, Seidahmed MZ, Kabiraj MM, Koenig M. Rundataxin, a novel protein with RUN and diacylglycerol binding domains, is mutant in a new recessive ataxia. *Brain* 2010; **133**: 2439-2447 [PMID: 20826435 DOI: 10.1093/brain/awq181]
108 **Vermeer S**, Hoischen A, Meijer RP, Gilissen C, Neveling K, Wieskamp N, de Brouwer A, Koenig M, Anheim M, Assoum M, Drouot N, Todorovic S, Milic-Rasic V, Lochmüller H, Stevanin G, Goizet C, David A, Durr A, Brice A, Kremer B, van de Warrenburg BP, Schijvenaars MM, Heister A, Kwint M, Arts P, van der Wijst J, Veltman J, Kamsteeg EJ, Scheffer H, Knoers N. Targeted next-generation sequencing of a 12.5 Mb homozygous region reveals ANO10 mutations in patients with autosomal-recessive cerebellar ataxia. *Am J Hum Genet* 2010;**87**: 813-819 [PMID: 21092923 DOI: 10.1016/j.ajhg.2010.10.015]
109 **Breedveld GJ**, van Wetten B, te Raa GD, Brusse E, van Swieten JC, Oostra BA, Maat-Kievit JA. A new locus for a childhood onset, slowly progressive autosomal recessive spinocerebellar ataxia maps to chromosome 11p15. *J Med Genet* 2004; **41**: 858-866 [PMID: 15520412]
110 **Sun Y**, Almomani R, Breedveld GJ, Santen GW, Aten E, Lefeber DJ, Hoff JI, Brusse E, Verheijen FW, Verdijk RM, Kriek M, Oostra B, Breuning MH, Losekoot M, den Dunnen JT, van de Warrenburg BP, Maat-Kievit AJ. Autosomal recessive spinocerebellar ataxia 7 (SCAR7) is caused by variants in TPP1, the gene involved in classic late-infantile neuronal ceroid lipofuscinosis 2 disease (CLN2 disease). *Hum Mutat* 2013; **34**: 706-713 [PMID: 23418007 DOI: 10.1002/humu.22292]
111 **Hammer MB**, Eleuch-Fayache G, Schottlaender LV, Nehdi H, Gibbs JR, Arepalli SK, Chong SB, Hernandez DG, Sailer A, Liu G, Mistry PK, Cai H, Shrader G, Sassi C, Bouhlal Y, Houlden H, Hentati F, Amouri R, Singleton AB. Mutations in GBA2 cause autosomal-recessive cerebellar ataxia with spasticity. *Am J Hum Genet* 2013; **92**: 245-251 [PMID: 23332917 DOI: 10.1016/j.ajhg.2012.12.012]
112 **Martin E**, Schüle R, Smets K, Rastetter A, Boukhris A, Loureiro JL, Gonzalez MA, Mundwiller E, Deconinck T, Wessner M, Jornea L, Oteyza AC, Durr A, Martin JJ, Schöls L, Mhiri C, Lamari F, Züchner S, De Jonghe P, Kabashi E, Brice A, Stevanin G. Loss of function of glucocerebrosidase GBA2 is responsible for motor neuron defects in hereditary spastic paraplegia. *Am J Hum Genet* 2013; **92**: 238-244 [PMID: 23332916 DOI: 10.1016/j.ajhg.2012.11.021]
113 **Yildiz Y**, Matern H, Thompson B, Allegood JC, Warren RL, Ramirez DM, Hammer RE, Hamra FK, Matern S, Russell DW. Mutation of beta-glucosidase 2 causes glycolipid storage disease and impaired male fertility. *J Clin Invest* 2006; **116**: 2985-2994 [PMID: 17080196 DOI: 10.1172/JCI29224]
114 **Mencarelli C**, Martinez-Martinez P. Ceramide function in the brain: when a slight tilt is enough. *Cell Mol Life Sci* 2013; **70**: 181-203 [PMID: 22729185 DOI: 10.1007/s00018-012-1038-x]
115 **Guergueltcheva V**, Azmanov DN, Angelicheva D, Smith KR, Chamova T, Florez L, Bynevelt M, Nguyen T, Cherninkova S, Bojinova V, Kaprelyan A, Angelova L, Morar B, Chandler D, Kaneva R, Bahlo M, Tournev I, Kalaydjieva L. Autosomal-recessive congenital cerebellar ataxia is caused by mutations in metabotropic glutamate receptor 1. *Am J Hum Genet* 2012; **91**: 553-564 [PMID: 22901947 DOI: 10.1016/j.ajhg.2012.07.019]

116 **Pierson TM**, Adams D, Bonn F, Martinelli P, Cherukuri PF, Teer JK, Hansen NF, Cruz P, Mullikin For The Nisc Comparative Sequencing Program JC, Blakesley RW, Golas G, Kwan J, Sandler A, Fuentes Fajardo K, Markello T, Tifft C, Blackstone C, Rugarli EI, Langer T, Gahl WA, Toro C. Whole-exome sequencing identifies homozygous AFG3L2 mutations in a spastic ataxia-neuropathy syndrome linked to mitochondrial m-AAA proteases. *PLoS Genet* 2011; **7**: e1002325 [PMID: 22022284 DOI: 10.1371/journal.pgen.1002325]
117 **Mariotti C**, Brusco A, Di Bella D, Cagnoli C, Seri M, Gellera C, Di Donato S, Taroni F. Spinocerebellar ataxia type 28: a novel autosomal dominant cerebellar ataxia characterized by slow progression and ophthalmoparesis. *Cerebellum* 2008; **7**: 184-188 [PMID: 18769991 DOI: 10.1007/s12311-008-0053-9]
118 **Cagnoli C**, Stevanin G, Brussino A, Barberis M, Mancini C, Margolis RL, Holmes SE, Nobili M, Forlani S, Padovan S, Pappi P, Zaros C, Leber I, Ribai P, Pugliese L, Assalto C, Brice A, Migone N, Dürr A, Brusco A. Missense mutations in the AFG3L2 proteolytic domain account for ~1.5% of European autosomal dominant cerebellar ataxias. *Hum Mutat* 2010; **31**: 1117-1124 [PMID: 20725928 DOI: 10.1002/humu.21342]
119 **Casari G**, De Fusco M, Ciarmatori S, Zeviani M, Mora M, Fernandez P, De Michele G, Filla A, Cocozza S, Marconi R, Dürr A, Fontaine B, Ballabio A. Spastic paraplegia and OXPHOS impairment caused by mutations in paraplegin, a nuclear-encoded mitochondrial metalloprotease. *Cell* 1998; **93**: 973-983 [PMID: 9635427 DOI: 10.1016/S0092-8674(00)81203-9]

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MINIREVIEWS

# Pure motor stroke as the most frequent lacunar syndrome: A clinical update

Adrià Arboix, María José Sánchez, Josep Lluís Martí-Vilalta

Adrià Arboix, Cerebrovascular Division, Department of Neurology, Hospital Universitari del Sagrat Cor, Universitat de Barcelona, E-08029 Barcelona, Catalonia, Spain
María José Sánchez, Medical Library, Hospital Universitari Sagrat Cor, E-08029 Barcelona, Catalonia, Spain
Josep Lluís Martí-Vilalta, Acute Stroke Unit, Department of Neurology, Hospital de la Santa Creu i Sant Pau, Universitat Autònoma de Barcelona, E-08026 Barcelona, Catalonia, Spain
Author contributions: Arboix A is the principal investigator, designed the minireview and wrote the paper, is also the corresponding author; Sánchez MJ contributed to write the paper, conducted the literature review, edited the manuscript and provided editorial assistance; Martí-Vilalta JL participated in the revision of the manuscript for intellectual content; All have read and approved the final version to be published.
Correspondence to: Adrià Arboix, MD, PhD, Cerebrovascular Division, Department of Neurology, Hospital Universitari del Sagrat Cor, Universitat de Barcelona, Viladomat 288, E-08029 Barcelona, Catalonia, Spain. aarboix@hscor.com
Telephone: +34–934948940 Fax: +34–934948906
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## Abstract

Pure motor stroke (PMS), also known as pure motor hemiparesis, is the most common of any lacunar form (between one half and two thirds of cases, depending on the series). In an acute stroke registry, 733 patients presented a lacunar infarct and PMS accounted for 12.7% ($n$ = 342) of all first-ever stroke patients and for 48% of all lacunar syndromes. The posterior limb of the internal capsule, corona radiata, and pons are the most frequent brain topographies. Infarcts in the mesencephalus or medullary pyramid have been exceptionally reported. This present update is focused on the clinical evidence and mechanisms underlying the relationship between PMS and different stroke etiologies.

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**Key words:** Pure motor stroke; Lacunar syndromes; Lacunar stroke; Cerebrovascular diseases; Stroke

**Core tip:** Pure motor stroke (PMS) is the most common of any lacunar form (between one half and two thirds of cases). The posterior limb of the internal capsule, corona radiata, and pons are the most frequent brain topographies. This present update is focused on the clinical evidence and mechanisms underlying the relationship between PMS and different stroke etiologies.

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## CLINICAL DEFINITION

Pure motor stroke (PMS), also known as pure motor hemiparesis, was first reported by Fisher and Curry in 1965 and it is considered the commonest lacunar syndrome in clinical practice, accounting for between one half and two thirds of cases, depending on the series. Lacunar infarction accounts for one fourth of all cerebral infarctions[1,2] and generally develops in patients with hypertension and/or diabetes mellitus.

Definition of known pre-stroke hypertension includes patients having systolic and diastolic blood pressure higher than 140/90 mmHg, respectively, measured at least twice by the general practitioner before stroke onset, or those with high blood pressure at the time of stroke onset with one or more of the following: hypertensive retinopathy, left ventricular hypertrophy diagnosed by electrocardiographic or echocardiographic criteria, or abnormal renal function (excluding diabetes or other alternative cause of nephropathy). In accordance with World Health Organization criteria, diabetes is diagnosed

**Table 1 Demographic characteristics and frequency of lacunar syndromes in the Sagrat Cor Hospital of Barcelona Stroke Registry**

| | *n* (%) |
|---|---|
| Total patients | 733 |
| Male | 423 (57.7) |
| Age, yr, mean (SD) | 74.1 (10.2) |
| Age ⩾ 85 years old | 110 (15.0) |
| Lacunar syndromes | |
| PMS | 352 (48) |
| Pure sensory stroke | 127 (17.4) |
| Sensorimotor stroke | 83 (11.3) |
| Dysartria-clumsy hand | 59 (8) |
| Ataxic-hemiparesis | 24 (3.3) |
| Atypical lacunar syndromes | 88 (12) |

PMS: Pure motor stroke.

**Table 3 Topographies in pure motor stroke**

| |
|---|
| Internal capsule |
| Pons |
| Corona radiata/centrum semiovale |
| Basal ganglia |
| Mesencepahalon |
| Medullary pyramid |
| Cerebral cortex[1] |

[1]Cerebral topography related to PMS not due to lacunar infarct. PMS: Pure motor stroke.

**Table 2 Distribution of weakness related to pure motor stroke in 128 patients included in the Sagrat Cor Hospital of Barcelona Stroke Registry**

| Distribution of weakness | PMS (*n* = 128) *n* (%) |
|---|---|
| Face, arm, leg | 112 (76) |
| Face, arm | 6 (4) |
| Arm, leg | 16 (10) |
| Face[1] | 6 (4) |
| Arm | 4 (3) |
| Leg | 4 (3) |

[1]Patients with isolated pure facial central palsy with dysarthria have been included in the atypical lacunar syndrome in another study. Modified from Arboix *et al*[6]. PMS: Pure motor stroke.

when post-stroke repeated fasting plasma glucose levels exceeded 7.8 mmol/L (149 mg/dL). Serum determination of HbA1c is useful in doubtful cases to diagnose previous diabetes.

Pierre Marie in 1901 and Ferrand in 1902 had first stressed the link between isolated sudden hemiparesis and lacunes.

In the Sagrat Cor Hospital of Barcelona Stroke Registry, PMS accounted for 48% of all lacunar syndromes[3] (Table 1). It was the first lacunar syndrome recognized clinically, and its features have been the most thoroughly explored[1]. The clinical criteria of PMS include the presence of unilateral partial or complete paresis involving at least two of three areas (face, upper limb, or lower limb) of the body and no evidence of aphasia, apraxia, and agnosia, nor visual field defect, eye movement disturbance, ataxia, sensory loss or evidence of bilateral weakness[4-6]. Pure motor monoparesis is almost never due to lacunar infarct (Table 2). These restricted motor deficits are more likely to be of cortical origin[2,6].

Although the main elements of the PMS syndromes are motor, other complaints are not rare, especially sensory disturbances, which occur initially in as many as 42% of cases[6]. These complaints usually present as numbness, heaviness, and loss of feeling. Only scan abnormalities are found on clinical examination. Improvement is seen in a high percentage of cases of PMS. Recovery is usually more rapid and more complete than that following cerebral surface infarction with a similar initial motor deficit[7]. The syndromes of partial hemiparesis show the best prognosis, as do those with the smaller infarct size on computed tomography scan or brain magnetic resonance imaging, but cases of complete hemiplegia followed by almost full recovery have been encountered[5,8].

Lacunar infarctions are not a benign vascular condition; in contrast, they present a high risk of recurrence and vascular dementia in the mid and long term[9-11].

Recurrent lacunar ischemic stroke may be associated with a more severe clinical picture and it is one of the major factors involved in producing lacunar state and vascular subcortical dementia[7-12]. Recurrent strokes are more likely to be lacunar if the index event is lacunar[5].

## CLINICO-ANATOMIC CORRELATIONS

PMS has been reported from autopsied cases as a focal small (< 20 mm in diameter), deep infarction (lacunar infarct subtype) involving the internal capsule, corona radiate, pons and medullary pyramid[1,2]. Other topographies are centrum semiovale, basal ganglia and mesencephalon (Table 3). The most common correlations have been with capsular locations. The greater number of lacunes has been reported in the posterior limb of internal capsule[5].

These anatomical structures are supplied by the lenticulostriate branches and the long deep perforator medullary branches of the anterior and middle cerebral arteries, and the paramedian branches of the basilar artery[1].

Small vessel diseases are mainly of atherosclerosis-degenerative type, such as lipohyalinosis or microatheromatosis of lacunar infarcts[1,2].

## OTHER CAUSES OF PURE MOTOR SYNDROMES

The concept of lacunar hypothesis (the presence of a lacunar syndrome is usually due to a lacunar infarct) suggested by Miller Fisher is clinically valid and useful in PMS[13-15]. This lacunar syndrome is commonly due to lacunar infarct resulting from small vessel disease. In a clinical study[5] of 222 patients with PMS, lacunar infarcts were found in 189 (85%) patients, whereas ischemic lacunar syndromes not due to lacunar infarcts occurred in 23 (10.4%) (atherothrombotic stroke in 12, cardioembolic

**Table 4 Pure motor stroke not due to lacunar infarcts**

| |
|---|
| Non-lacunar ischemic stroke[1] |
| Cardioembolic stroke |
| Atherothrombotic infarct |
| Internal carotid artery occlusion in the neck |
| Ventromedial pontine infarction due to a propagating thrombosis of the basilar branch |
| Complex aortic atheroma plaques |
| Ischemic stroke of unusual etiology |
| Ischemia-edema after craniotomy for postoperative bleeding |
| Intracerebral hemorrhage |
| Subdural hematoma |
| Brain abscess of the motor cortex |
| Cerebral metastasis |
| Demyelinating disease |

[1]Cerebral cortical surface infarction or large deep infarction (> 20 mm diameter).

stroke in 7, infarction of undetermined origin in 3 and infarction of unusual etiology in one) and hemorrhagic lacunar syndromes in 10 (4.5%). Other causes of PMS[14,16] are showed in Table 4. These rare causes can nowadays be easily detected by imaging techniques.

However, the lacunar syndromes do not predict lacunar infarcts when used in the first hours of stroke. Later, they have a reasonable predictive power if the classic definition is retained[17].

## PURE MOTOR STROKE OF UNUSUAL ETIOLOGY

PMS and the other lacunar strokes may be caused in less than 5% of cases by other etiologies, mainly hematological diseases and infectious or inflammatory arteritis[4,5,18].

Hematological disorders associated with lacunar stroke include polycythemia vera, essential thrombocythemia, and primary antiphospholipid antibody syndrome[18].

Infectious arteritis due to neurosyphilis, neurocysticercosis, neuroborreliosis, and acquired immunodeficiency syndrome has been also associated with lacunar infarction. In patients with first ever stroke, chronic *Helicobacter pylori* infection detected by IgG antibodies is associated with risk of small artery occlusion[5].

Moreover, inflammatory arteritis in systemic lupus erythematosus or granulomatous angiitis and polyarteritis nodosa have been related to lacunar infarctions, although in cases of polyarteritis nodosa, lacunar strokes are due to thrombotic microangiopathy, not vasculitis[18].

Drug abuse, particularly of cocaine, and pseudoxanthoma elasticum may produce small, deep infarcts[5].

Other uncommon etiologies include hereditary (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy), mitochondrial (mitochondrial encephalopathy, lactic acidosis, and stroke-like episodes), deposition of substances in the vessel wall (amyloid in sporadic and hereditary cerebral amyloid angiopathy or ceramide in Fabry's disease), or toxemic cause. Other small vessel diseases are venous collagenosis, post-radiation angiopathy and non-amyloid microvessel degeneration in Alzheimer disease[5,18].

## CONCLUSION

PMS is the most common lacunar syndrome. The lacunar hypothesis is clinically valid and useful in PMS; however in 10%-15% of the cases PMS may be associated with underlying non-lacunar ischemic mechanisms such as cardiac source of embolism, atherosclerosis, intracerebral hemorrhage or cerebral ischemia of unusual etiology that may influence management. Thus, it is mandatory to establish the etiological diagnosis of PMS to classify correctly the stroke subtype.

## REFERENCES


1 **Fisher CM**, Curry HB. Pure motor hemiplegia of vascular origin. *Arch Neurol* 1965; **13**: 30-44 [PMID: 14314272 DOI: 10.1001/archneur.1965.00470010034005]

2 **Fisher CM**. Lacunar infarcts. A review. *Cerebrovasc Dis* 1991; **1**: 311–320 [DOI: 10.1159/000108861]

3 **Arboix A**, Padilla I, García-Eroles, Massons J, Comes E, Targa C. Pure motor hemiparesis: a clinical study of 222 patients. *J Neurol Neurosurg Psychiatry* 2001; **71**: 239–242 [PMID: 11459902 DOI: 10.1136/jnnp.71.2.239]

4 **Chamorro A**, Sacco RL, Mohr JP, Foulkes MA, Kase CS, Tatemichi TK, Wolf PA, Price TR, Hier DB. Clinical-computed tomographic correlations of lacunar infarction in the Stroke Data Bank. *Stroke* 1991; **22**: 175-181 [PMID: 2003281 DOI: 10.1161/01.STR.22.2.175]

5 **Arboix A**, Martí-Vilalta JL. Lacunar stroke. *Expert Rev Neurother* 2009; **9**: 179-196 [PMID: 19210194 DOI: 10.1586/14737175.9.2.179]

6 **Arboix A**, Martí-Vilalta JL, García JH. Clinical study of 227 patients with lacunar infarcts. *Stroke* 1990; **21**: 842-847 [PMID: 2349585 DOI: 10.1161/01.STR.21.6.842]

7 **Pantoni L**. Cerebral small vessel disease: from pathogenesis and clinical characteristics to therapeutic challenges. *Lancet Neurol* 2010; **9**: 689-701 [PMID: 20610345 DOI: 10.1016/S1474-4422(10)70104-6]

8 **Del Bene A**, Palumbo V, Lamassa M, Saia V, Piccardi B, Inzitari D. Progressive lacunar stroke: review of mechanisms, prognostic features, and putative treatments. *Int J Stroke* 2012; **7**: 321-329 [PMID: 22463492 DOI: 10.1111/j.1747-4949.2012.00789.x]

9 **Jacova C**, Pearce LA, Costello R, McClure LA, Holliday SL, Hart RG, Benavente OR. Cognitive impairment in lacunar strokes: the SPS3 trial. *Ann Neurol* 2012; **72**: 351-362 [PMID: 23034910 DOI: 10.1002/ana.23733]

10 **Grau-Olivares M**, Bartrés-Faz D, Arboix A, Soliva JC, Rovira M, Targa C, Junqué C. Mild cognitive impairment after lacunar infarction: voxel-based morphometry and neuropsychological assessment. *Cerebrovasc Dis* 2007; **23**: 353-361 [PMID: 17268166 DOI: 10.1159/000099134]

11 **The SPS3 Investigators**, Benavente OR, Hart RG, McClure LA, Szychowski JM, Coffey CS, Pearce LA. Effects of clopidogrel added to aspirin in patients with recent lacunar stroke. *N Engl J Med* 2012; **367**: 817-825 [PMID: 22931315 DOI: 10.1056/NEJMoa1204133]

12 **Arboix A**, Font A, Garro C, García-Eroles L, Comes E, Massons J. Recurrent lacunar infarction following a previous lacunar stroke: a clinical study of 122 patients. *J Neurol Neurosurg Psychiatry* 2007; **78**: 1392-1394 [PMID: 17615167 DOI: 10.1136/jnnp.2007.119776]

13 **Arboix A**, Massons J, García-Eroles L, Targa C, Comes E, Parra O. Clinical predictors of lacunar syndrome not due to lacunar infarcts. *BMC Neurol* 2010; **10**: 31 [PMID: 20482763

DOI: 10.1186/1471-2377-10-31]
14 **Arboix A**, Martí-Vilalta JL. Lacunar syndromes not due to lacunar infarcts. *Cerebrovasc Dis* 1992; **2**: 287–292 [DOI: 10.1159/000109029]
15 **Micheli S**, Agnelli G, Palmerini F, Caso V, Venti M, Alberti A, Biagini S, Paciaroni M. Need for extensive diagnostic work-up for patients with lacunar stroke. *J Neurol* 2008; **255**: 637-642 [PMID: 18283395 DOI: 10.1007/s00415-008-0762-5]
16 **Arboix A**, García-Eroles L, Massons J, Oliveres M, Targa C. Hemorrhagic lacunar stroke. *Cerebrovasc Dis* 2000; **10**: 229-234 [PMID: 10773650 DOI: 10.1159/000016061]
17 **Ferro JM**. Patterns of ischaemic cerebral diseases. *J Neurol* 2004; **251**: 1-10 [PMID: 14999483 DOI: 10.1007/s00415-004-0280-z]
18 **Arboix A**, Martí-Vilalta JL. New concepts in lacunar stroke etiology: the constellation of small-vessel arterial disease. *Cerebrovasc Dis* 2004; **17** Suppl 1: 58-62 [PMID: 14694281 DOI: 10.1159/000074796]

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MINIREVIEWS

# Overview of botulinum toxin as a treatment for spasticity in stroke patients

Hirotaka Isoyama, Naoyuki Takeuchi

Hirotaka Isoyama, Department of Physical Medicine and Rehabilitation, Hospital of Hokkaido University, Hokkaido 060-8648, Japan
Naoyuki Takeuchi, Department of Physical Medicine and Rehabilitation, Tohoku University Graduate School of Medicine, Sendai 980-8576, Japan
Author contributions: Both authors contributed to the manuscript.
Correspondence to: Hirotaka Isoyama, MD, Department of Physical Medicine and Rehabilitation, Hospital of Hokkaido University, Kita14 Nishi5 Kita-ku, Sapporo, Hokkaido 060-8648, Japan. isoyama@huhp.hokudai.ac.jp
Telephone: +81-11-7066066 Fax: +81-11-7066067
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## Abstract

Spasticity after the occurrence of stroke induces limb deformity, functional disability and/or pain in patients, which limits their activities of daily living and deteriorates their quality of life. Botulinum toxin (BTX) has recently been reported as an efficacious therapeutic agent for the treatment of spasticity. Systematic review and meta-analysis studies have demonstrated that BTX therapy after stroke reduces spasticity and increases physical activity capacity and performance levels. Moreover, BTX can be used as an adjuvant in physiotherapy. Several studies have confirmed that the combination of BTX therapy and physiotherapy improves motor recovery. However, to date, only a few such combination studies have been conducted and their findings are considered preliminary and controversial. Therefore, future studies are required to determine the appropriate combination of treatment methods that will aid motor recovery.

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**Key words:** Botulinum toxin; Rehabilitation; Stroke; Spasticity; Neurorehabilitation

**Core tip:** In this overview, we describe that botulinum toxins (BTXs) were very useful to reduce spasticity in stroke patients. Moreover, we introduce studies on appropriate effective and safe injection methods; pilot studies that evaluated combined rehabilitation and BTX treatment; and adverse events after BTX injection, including risk factors of neutralizing antibodies.

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## INTRODUCTION

Spasticity is a common complication in patients who have experienced stroke. The incidence of spasticity is reported to be 17%-43% in post-stroke patients[1-3]. Spasticity induces limb deformity, functional disability and/or pain that limits activities of daily living (ADL) and deteriorates the quality of life (QOL). In order to reduce spasticity, clinicians prescribe oral medications, intrathecal baclofen therapy, nerve phenolization, casting or splinting, rehabilitation therapy or a combination of these therapeutic approaches[4-8]. A recent study has shown that botulinum toxin (BTX) is an efficacious therapeutic agent for the treatment of spasticity[9].

BTX is poisonous in nature and is produced by the anaerobic bacterium *Clostridium Botulinum*[10]. BTX blocks vesicular acetylcholine release at neuromuscular junctions and reduces focal muscle tonus and contracture[11]. Therefore, BTX is used for treating muscular hyperactivity and excessive or inappropriate muscle contraction. Seven types of BTX exist in nature, but two toxin types, type A (BTX-A) and type B (BTX-B), are used in the clinical

setting. Most clinical trials have utilized BTX-A because it has a longer lasting effect than BTX-B[12,13]. BTX-B tends to be selected for spasticity treatment when patients have neutralizing antibodies against BTX-A or develop antibodies after repetitive BTX-A injection treatment[14,15].

Many studies have reported that BTX injection treatment reduces spasticity in stroke patients[16-25]. However, a few studies have focused on rehabilitation therapy after BTX treatment for improving motor function[26-28]. This review focuses on the following factors of BTX treatment in post-stroke patients: (1) the effectiveness of spasticity reduction, with regard to ADL and QOL; (2) the appropriate injection method; (3) combined BTX therapy and neurorehabilitation; and (4) adverse events. The purpose of this review is to provide a comprehensive overview of BTX treatment for muscular spasticity in post-stroke patients, to understand its mechanisms of action, and to suggest appropriate treatment approaches.

## EFFECTIVENESS FOR SPASTICITY

Several reports clarified the effectiveness of BTX treatment for upper limb spasticity experienced after stroke[16-21]. A recent systematic review and meta-analysis reported that BTX-A reduced spasticity and thereby improved physical activity capacity and performance levels in the upper limb in patients who experienced stroke[29]. BTX-A (Botox®) injection in the upper limb muscles are recommended at the following doses: 25-100 units for the flexor carpi radialis; 20-70 units for the flexor carpi ulnaris; 20-60 units for the flexor digitorum superficialis; 20-60 units for the flexor digitorum profundus; 10-30 units for the flexor pollicis longus; and 5-25 units for the adductor pollicis[30].

Furthermore, several studies have reported the effectiveness of BTX therapy in stroke-induced lower limb spasticity[22-25]. However, a systematic review and meta-analysis reported that BTX-A treatment for stroke-induced lower limb spasticity was associated with a slight improvement in functional ambulation[19,31]. A multicenter, double-blind trial demonstrated the effectiveness of BTX-A in cases of lower limb spasticity, but improvements in gait pattern scale and speed were not significant, compared to that observed in the placebo group[32]. A previous study suggested that high-dose BTX-A treatment may induce excessive muscle weakening that may deteriorate limb function[25]. BTX-A (Botox®) injection doses in the lower limb is recommended at the following doses: 50-250 units for the medial and lateral head of the gastrocnemius respectively; 50-200 units for the soleus; and 50-150 units for the tibialis posterior[30]. However, to the best of our knowledge, no study has evaluated the appropriate BTX dose for treatment of upper and lower limb spasticity and therefore, future studies are required to clarify these dosages. Moreover, it is noted that clinicians should consider the maximum dose of BTX per session, which differs in each country (*e.g.,* 360 units in Japan and 600 units in Europe)[32-35].

Treatment of spasticity in patients who experience a stroke with BTX-A may improve ADL and QOL[36,37]. A previous study reported that high-dose BTX-A significantly improved ADL scores for limb position and self-dressing[36]. Furthermore, another study in post-stroke patients reported that BTX-A therapy improved overall QOL scores and muscle tone, reduced disability, and improved the ability to function[37]. However, a recent meta-analysis study revealed that BTX-A did not significantly improve overall health-related QOL[38].

## APPROPRIATE INJECTION METHOD

Several studies have reported on BTX-A injection techniques[39-41]. Francisco *et al*[39] compared the efficacy of high and low volumes of BTX at the same dose for wrist and finger flexor spasticity. They found that modified Ashworth Scale scores were significantly decreased in both high and low-volume injection groups. However, there was no significant difference between the groups[40]. Gracies *et al*[41] reported that injecting a small volume of BTX-A near the neuromuscular junction was more effective than injecting a similar volume at a greater distance from the neuromuscular junction. However, injecting a large volume at a greater distance from the junction was as effective as a small volume injected near the neuromuscular junction. Mayer *et al*[40] also reported that a single motor point and multisite low-dose, high-volume BTX-A injections produced a similar impact in spasticity reduction. Therefore, the appropriate injection methods according to BTX-A volume and injection points must be elucidated. Moreover, BTX-A injections are more accurate when the affected muscles are targeted *via* needle electromyography or under ultrasound guidance. This conventional method is not supported by evidence, but correct muscle selection has been confirmed to be a key feature in the efficacy of BTX treatment[42,43].

## COMBINATION OF BTX THERAPY AND NEUROREHABILITATION

As mentioned above, BTX improves motor function by reducing muscular spasticity in post-stroke patients. Therefore, BTX therapy may be a useful adjuvant therapy in combination with neurorehabilitation. In fact, several studies have reported that the combination of BTX therapy and physiotherapy improved motor recovery[26-28]. A recent study reported that BTX injection, followed by home-based functional training, may have the potential to improve active motor function of the affected upper limb in post-stroke patients[26]. Moreover, previous studies have reported that casting and dynamic splinting following BTX-A injection improved range of motion as well as motor function[5,27,28]. Thus, BTX when combined with physiotherapy or casting may be useful for improving motor function in stroke-induced spasticity.

Recently, various neurorehabilitation strategies have been established to improve neural plasticity and motor recovery. These approaches include constraint-induced

movement therapy (CIMT), transcutaneous electrical neuromuscular stimulation (TENS) and non-invasive brain stimulation (NIBS)[44-49]. A preliminary study demonstrated that the combination of BTX injection and CIMT may improve functional gains in post-stroke patients[44,45]. Moreover, a recent study reported that combined BTX-A and NIBS improves motor function in patients with stroke-induced upper limb hemiparesis[48]. However, a previous study reported that combining BTX injection with TENS did not have any additional effect on motor recovery[47]. The number of these combination studies remains small and their results are considered preliminary and controversial. Although BTX-A probably enhances the effects of neurorehabilitation strategies in post-stroke patients, future studies are required to determine the appropriate combination of treatments for optimum motor recovery.

## ADVERSE EVENTS

In general, pain, rash, edema and muscle weakness have been noted at the injection site and remote muscle weakness, fatigue and allergic reaction have been reported as adverse events related to BTX therapy. To clarify the safety of BTX-A, Turkel *et al*[50] analyzed 9 double-blind, placebo-controlled studies in more than 500 patients who had experienced a stroke. The most frequent reported adverse events by the patients (> 5% but < 10% in either group) were respiratory infection, seizures, incoordination and injection site pain, none of which occurred at a significantly high rate in the BTX-A group. Nausea alone was reported at a significantly higher rate in the BTX-A group than in the placebo group. In contrast, injection site pain, chest pain and allergic reaction were found at a significantly higher frequency in the placebo group. Other reviews have also reported no serious adverse events after BTX-A administration in adult post-stroke patients[35]. Therefore, BTX has recently been considered a safe and efficacious treatment for spasticity in post-stroke patients.

However, care should be taken to try to avoid producing neutralizing antibodies. A higher dose per treatment, repetitive treatment and shorter intervals between treatments are risk factors for the production of neutralizing antibodies[51-53]. Of these, the interval between treatments is the most important risk factor. Therefore, BTX-A treatment intervals of more than three months and BTX-B treatment intervals of two months are recommended[54].

## CONCLUSION

Many reports have shown the usefulness of the BTX treatment for spasticity in stroke patients. However, a few studies have reported the effectiveness of rehabilitation after BTX treatment in stroke patients. Future studies are needed to evaluate the efficacy of BTX after treatment with rehabilitation.

## REFERENCES


1 **Sommerfeld DK**, Eek EU, Svensson AK, Holmqvist LW, von Arbin MH. Spasticity after stroke: its occurrence and association with motor impairments and activity limitations. *Stroke* 2004; **35**: 134-139 [PMID: 14684785 DOI: 10.1161/01.STR.0000105386.05173.5E]

2 **Lundström E**, Terént A, Borg J. Prevalence of disabling spasticity 1 year after first-ever stroke. *Eur J Neurol* 2008; **15**: 533-539 [PMID: 18355307 DOI: 10.1111/j.1468-1331.2008.02114.x]

3 **Urban PP**, Wolf T, Uebele M, Marx JJ, Vogt T, Stoeter P, Bauermann T, Weibrich C, Vucurevic GD, Schneider A, Wissel J. Occurence and clinical predictors of spasticity after ischemic stroke. *Stroke* 2010; **41**: 2016-2020 [PMID: 20705930 DOI: 10.1161/STROKEAHA.110.581991]

4 **Ward AB.** A summary of spasticity management--a treatment algorithm. *Eur J Neurol* 2002; **9** Suppl 1**:** 48-52; dicussion 53-61 [PMID: 11918650 DOI: 10.1046/j.1468-1331.2002.0090s1048.x]

5 **Farina S**, Migliorini C, Gandolfi M, Bertolasi L, Casarotto M, Manganotti P, Fiaschi A, Smania N. Combined effects of botulinum toxin and casting treatments on lower limb spasticity after stroke. *Funct Neurol* 2008; **23**: 87-91 [PMID: 18671909]

6 **Reiter F**, Danni M, Lagalla G, Ceravolo G, Provinciali L. Low-dose botulinum toxin with ankle taping for the treatment of spastic equinovarus foot after stroke. *Arch Phys Med Rehabil* 1998; **79**: 532-535 [PMID: 9596394 DOI: 10.1016/S0003-9993(98)90068-5]

7 **Verplancke D**, Snape S, Salisbury CF, Jones PW, Ward AB. A randomized controlled trial of botulinum toxin on lower limb spasticity following acute acquired severe brain injury. *Clin Rehabil* 2005; **19**: 117-125 [PMID: 15759526]

8 **Richardson D**. Physical therapy in spasticity. *Eur J Neurol* 2002; **9** Suppl 1: 17-22; dicussion 53-61 [PMID: 11918645 DOI: 10.1191/0269215505cr827oa]

9 **Intiso D**. Therapeutic use of botulinum toxin in neurorehabilitation. *J Toxicol* 2012; **2012**: 802893 [PMID: 21941544 DOI: 10.1155/2012/802893]

10 **Das Gupta BR**, Sugiyama H. Role of a protease in natural activation of Clostridium botulinum neurotoxin. *Infect Immun* 1972; **6**: 587-590 [PMID: 4564288]

11 **Pearce LB**, First ER, MacCallum RD, Gupta A. Pharmacologic characterization of botulinum toxin for basic science and medicine. *Toxicon* 1997; **35**: 1373-1412 [PMID: 9403963 DOI: 10.1016/S0041-0101(96)00180-8]

12 **Keller JE**. Recovery from botulinum neurotoxin poisoning in vivo. *Neuroscience* 2006; **139**: 629-637 [PMID: 16490322 DOI: 10.1016/j.neuroscience.2005.12.029]

13 **Comella CL**, Jankovic J, Shannon KM, Tsui J, Swenson M, Leurgans S, Fan W. Comparison of botulinum toxin serotypes A and B for the treatment of cervical dystonia. *Neurology* 2005; **65**: 1423-1429 [PMID: 16275831 DOI: 10.1212/01.wnl.0000183055.81056.5c]

14 **Barnes MP**, Best D, Kidd L, Roberts B, Stark S, Weeks P, Whitaker J. The use of botulinum toxin type-B in the treatment of patients who have become unresponsive to botulinum toxin type-A -- initial experiences. *Eur J Neurol* 2005; **12**: 947-955 [PMID: 16324088 DOI: 10.1111/j.1468-1331.2005.01095.x]

15 **Factor SA**, Molho ES, Evans S, Feustel PJ. Efficacy and safety of repeated doses of botulinum toxin type B in type A resistant and responsive cervical dystonia. *Mov Disord* 2005; **20**: 1152-1160 [PMID: 15954134 DOI: 10.1002/mds.20531]

16 **Brashear A**, Gordon MF, Elovic E, Kassicieh VD, Marciniak C, Do M, Lee CH, Jenkins S, Turkel C. Intramuscular injection of botulinum toxin for the treatment of wrist and finger spasticity after a stroke. *N Engl J Med* 2002; **347**: 395-400 [PMID: 12167681 DOI: 10.1056/NEJMoa011892]

17 **Bakheit AM**, Pittock S, Moore AP, Wurker M, Otto S, Erbguth F, Coxon L. A randomized, double-blind, placebo-

controlled study of the efficacy and safety of botulinum toxin type A in upper limb spasticity in patients with stroke. *Eur J Neurol* 2001; **8**: 559-565 [PMID: 11784339 DOI: 10.1046/j.1468-1331.2001.00277.x]

18 **Simpson DM**, Alexander DN, O'Brien CF, Tagliati M, Aswad AS, Leon JM, Gibson J, Mordaunt JM, Monaghan EP. Botulinum toxin type A in the treatment of upper extremity spasticity: a randomized, double-blind, placebo-controlled trial. *Neurology* 1996; **46**: 1306-1310 [PMID: 8628472 DOI: 10.1212/WNL.46.5.1306]

19 **Rosales RL**, Chua-Yap AS. Evidence-based systematic review on the efficacy and safety of botulinum toxin-A therapy in post-stroke spasticity. *J Neural Transm* 2008; **115**: 617-623 [PMID: 18322637 DOI: 10.1007/s00702-007-0869-3]

20 **Cardoso E**, Rodrigues B, Lucena R, Oliveira IR, Pedreira G, Melo A. Botulinum toxin type A for the treatment of the upper limb spasticity after stroke: a meta-analysis. *Arq Neuropsiquiatr* 2005; **63**: 30-33 [PMID: 15830061 DOI: 10.1590/S0004-282X2005000100006]

21 **Yablon SA,** Brin MF, VanDenburgh AM, Zhou J, Garabedian-Ruffalo SM, Abu-Shakra S, Beddingfield FC 3rd. Dose response with onabotulinumtoxinA for post-stroke spasticity: a pooled data analysis. *Mov Disord* 2011; **26**: 209-215 [PMID: 20960474 DOI: 10.1002/mds.23426]

22 **Grazko MA**, Polo KB, Jabbari B. Botulinum toxin A for spasticity, muscle spasms, and rigidity. *Neurology* 1995; **45**: 712-717 [PMID: 7723960 DOI: 10.1212/WNL.45.4.712]

23 **Burbaud P**, Wiart L, Dubos JL, Gaujard E, Debelleix X, Joseph PA, Mazaux JM, Bioulac B, Barat M, Lagueny A. A randomised, double blind, placebo controlled trial of botulinum toxin in the treatment of spastic foot in hemiparetic patients. *J Neurol Neurosurg Psychiatry* 1996; **61**: 265-269 [PMID: 8795597 DOI: 10.1136/jnnp.61.3.265]

24 **Dunne J.** Botulinum toxin type A (BOTOX) in the treatment of lower limb spasticity during stroke rehabilitation. *Intern Med J* 2003; **33** (Suppl): A41-A54

25 **Mancini F**, Sandrini C, Moglia A, Nappi G, Pacchetti C. A randomised, double-blind, dose-ranging study to evaluate efficacy and safety of three doses of botulinum toxin type A (Botox) for the treatment of spastic foot. *Neurol Sci* 2005; **26**: 26-31 [PMID: 15877184 DOI: 10.1007/s10072-005-0378-9]

26 **Takekawa T**, Kakuda W, Taguchi K, Ishikawa A, Sase Y, Abo M. Botulinum toxin type A injection, followed by home-based functional training for upper limb hemiparesis after stroke. *Int J Rehabil Res* 2012; **35**: 146-152 [PMID: 22453625 DOI: 10.1097/MRR.0b013e3283527f4a]

27 **Yaşar E**, Tok F, Safaz I, Balaban B, Yilmaz B, Alaca R. The efficacy of serial casting after botulinum toxin type A injection in improving equinovarus deformity in patients with chronic stroke. *Brain Inj* 2010; **24**: 736-739 [PMID: 20334469 DOI: 10.3109/02699051003610524]

28 **Lai JM**, Francisco GE, Willis FB. Dynamic splinting after treatment with botulinum toxin type-A: a randomized controlled pilot study. *Adv Ther* 2009; **26**: 241-248 [PMID: 19194671 DOI: 10.1007/s12325-008-0139-2]

29 **Foley N**, Pereira S, Salter K, Fernandez MM, Speechley M, Sequeira K, Miller T, Teasell R. Treatment with botulinum toxin improves upper-extremity function post stroke: a systematic review and meta-analysis. *Arch Phys Med Rehabil* 2013; **94**: 977-989 [PMID: 23262381 DOI: 10.1016/j.apmr.2012.12.006]

30 **Francisco GE**. Botulinum toxin for post-stroke spastic hypertonia: a review of its efficacy and application in clinical practice. *Ann Acad Med Singapore* 2007; **36**: 22-30 [PMID: 17285183]

31 **Foley N**, Murie-Fernandez M, Speechley M, Salter K, Sequeira K, Teasell R. Does the treatment of spastic equinovarus deformity following stroke with botulinum toxin increase gait velocity? A systematic review and meta-analysis. *Eur J Neurol* 2010; **17**: 1419-1427 [PMID: 20491885 DOI: 10.1111/j.1468-1331.2010.03084.x]

32 **Kaji R**, Osako Y, Suyama K, Maeda T, Uechi Y, Iwasaki M. Botulinum toxin type A in post-stroke lower limb spasticity: a multicenter, double-blind, placebo-controlled trial. *J Neurol* 2010; **257**: 1330-1337 [PMID: 20358216 DOI: 10.1007/s00415-010-5526-3]

33 **Wissel J**, Ward AB, Erztgaard P, Bensmail D, Hecht MJ, Lejeune TM, Schnider P, Altavista MC, Cavazza S, Deltombe T, Duarte E, Geurts AC, Gracies JM, Haboubi NH, Juan FJ, Kasch H, Kätterer C, Kirazli Y, Manganotti P, Parman Y, Paternostro-Sluga T, Petropoulou K, Prempeh R, Rousseaux M, Slawek J, Tieranta N. European consensus table on the use of botulinum toxin type A in adult spasticity. *J Rehabil Med* 2009; **41**: 13-25 [PMID: 19197564 DOI: 10.2340/16501977-0303]

34 **Ozcakir S**, Sivrioglu K. Botulinum toxin in poststroke spasticity. *Clin Med Res* 2007; **5**: 132-138 [PMID: 17607049 DOI: 10.3121/cmr.2007.716]

35 **Elia AE**, Filippini G, Calandrella D, Albanese A. Botulinum neurotoxins for post-stroke spasticity in adults: a systematic review. *Mov Disord* 2009; **24**: 801-812 [PMID: 19224614 DOI: 10.1002/mds.22452]

36 **Kaji R**, Osako Y, Suyama K, Maeda T, Uechi Y, Iwasaki M. Botulinum toxin type A in post-stroke upper limb spasticity. *Curr Med Res Opin* 2010; **26**: 1983-1992 [PMID: 20569068 DOI: 10.1185/03007995.2010.497103]

37 **Elovic EP**, Brashear A, Kaelin D, Liu J, Millis SR, Barron R, Turkel C. Repeated treatments with botulinum toxin type a produce sustained decreases in the limitations associated with focal upper-limb poststroke spasticity for caregivers and patients. *Arch Phys Med Rehabil* 2008; **89**: 799-806 [PMID: 18452724 DOI: 10.1016/j.apmr.2008.01.007]

38 **Pulman J**, Buckley E, Clark-Carter D. A meta-analysis evaluating the effectiveness of two different upper limb hemiparesis interventions on improving health-related quality of life following stroke. *Top Stroke Rehabil* 2013; **20**: 189-196 [PMID: 23611859 DOI: 10.1310/tsr2002-189]

39 **Francisco GE**, Boake C, Vaughn A. Botulinum toxin in upper limb spasticity after acquired brain injury: a randomized trial comparing dilution techniques. *Am J Phys Med Rehabil* 2002; **81**: 355-363 [PMID: 11964576 DOI: 10.1097/00002060-200205000-00007]

40 **Mayer NH**, Whyte J, Wannstedt G, Ellis CA. Comparative impact of 2 botulinum toxin injection techniques for elbow flexor hypertonia. *Arch Phys Med Rehabil* 2008; **89**: 982-987 [PMID: 18452749 DOI: 10.1016/j.apmr.2007.10.022]

41 **Gracies JM**, Lugassy M, Weisz DJ, Vecchio M, Flanagan S, Simpson DM. Botulinum toxin dilution and endplate targeting in spasticity: a double-blind controlled study. *Arch Phys Med Rehabil* 2009; **90**: 9-16.e2 [PMID: 19154823 DOI: 10.1016/j.apmr.2008.04.030]

42 **Cordivari C**, Misra VP, Vincent A, Catania S, Bhatia KP, Lees AJ. Secondary nonresponsiveness to botulinum toxin A in cervical dystonia: the role of electromyogram-guided injections, botulinum toxin A antibody assay, and the extensor digitorum brevis test. *Mov Disord* 2006; **21**: 1737-1741 [PMID: 16874756 DOI: 10.1002/mds.21051]

43 **Schnitzler A**, Roche N, Denormandie P, Lautridou C, Parratte B, Genet F. Manual needle placement: accuracy of botulinum toxin A injections. *Muscle Nerve* 2012; **46**: 531-534 [PMID: 22987693 DOI: 10.1002/mus.23410]

44 **Levy CE**, Giuffrida C, Richards L, Wu S, Davis S, Nadeau SE. Botulinum toxin a, evidence-based exercise therapy, and constraint-induced movement therapy for upper-limb hemiparesis attributable to stroke: a preliminary study. *Am J Phys Med Rehabil* 2007; **86**: 696-706 [PMID: 17709993 DOI: 10.1097/PHM.0b013e31813e2b4d]

45 **Sun SF**, Hsu CW, Hwang CW, Hsu PT, Wang JL, Yang CL. Application of combined botulinum toxin type A and modified constraint-induced movement therapy for an individual with chronic upper-extremity spasticity after stroke. *Phys*

*Ther* 2006; **86**: 1387-1397 [PMID: 17012643 DOI: 10.2522/ptj.20050262]

46 **Santamato A**, Notarnicola A, Panza F, Ranieri M, Micello MF, Manganotti P, Moretti B, Fortunato F, Filoni S, Fiore P. SBOTE study: extracorporeal shock wave therapy versus electrical stimulation after botulinum toxin type a injection for post-stroke spasticity-a prospective randomized trial. *Ultrasound Med Biol* 2013; **39**: 283-291 [PMID: 23245824 DOI: 10.1016/j.ultrasmedbio.2012.09.019]

47 **Weber DJ,** Skidmore ER, Niyonkuru C, Chang CL, Huber LM, Munin MC. Cyclic functional electrical stimulation does not enhance gains in hand grasp function when used as an adjunct to onabotulinumtoxinA and task practice therapy: a single-blind, randomized controlled pilot study. *Arch Phys Med Rehabil* 2010; **91:** 679-686 [PMID: 20434603 DOI: 10.1016/J.apmr.2010.01.010]

48 **Kakuda W**, Abo M, Momosaki R, Yokoi A, Fukuda A, Ito H, Tominaga A, Umemori T, Kameda Y. Combined therapeutic application of botulinum toxin type A, low-frequency rTMS, and intensive occupational therapy for post-stroke spastic upper limb hemiparesis. *Eur J Phys Rehabil Med* 2012; **48**: 47-55 [PMID: 22071503 DOI: 10.1097/01.phm.0000193505.85874.61]

49 **Bayram S**, Sivrioglu K, Karli N, Ozcan O. Low-dose botulinum toxin with short-term electrical stimulation in post-stroke spastic drop foot: a preliminary study. *Am J Phys Med Rehabil* 2006; **85**: 75-81 [PMID: 16357552]

50 **Turkel CC**, Bowen B, Liu J, Brin MF. Pooled analysis of the safety of botulinum toxin type A in the treatment of post-stroke spasticity. *Arch Phys Med Rehabil* 2006; **87**: 786-792 [PMID: 16731213 DOI: 10.1016/j.apmr.2006.02.015]

51 **Dressler D**. Clinical features of antibody-induced complete secondary failure of botulinum toxin therapy. *Eur Neurol* 2002; **48**: 26-29 [PMID: 12138306 DOI: 10.1159/000064953]

52 **Greene P**, Fahn S, Diamond B. Development of resistance to botulinum toxin type A in patients with torticollis. *Mov Disord* 1994; **9**: 213-217 [PMID: 8196686 DOI: 10.1002/mds.870090216]

53 **Hatheway CL,** Dang C. Immunogenicity of the neurotoxins of Clostridium botulinum. In: Jankovic J, Hallett M, eds. Therapy with Botulinum Toxin. New York: Marcel Dekker, 1994: 93-107

54 **Varghese-Kroll E**, Elovic EP. Contralateral weakness and fatigue after high-dose botulinum toxin injection for management of poststroke spasticity. *Am J Phys Med Rehabil* 2009; **88**: 495-499 [PMID: 19454855 DOI: 10.1097/PHM.0b013e3181a5b056]

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MINIREVIEWS

# Neuritin: A therapeutic candidate for promoting axonal regeneration

Tadayuki Shimada, Hiroko Sugiura, Kanato Yamagata

Tadayuki Shimada, Hiroko Sugiura, Kanato Yamagata, Neural Plasticity Project, Tokyo Metropolitan Institute of Medical Science, Setagaya-ku, Tokyo 156-8506, Japan
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Correspondence to: Kanato Yamagata, MD, Neural Plasticity Project, Tokyo Metropolitan Institute of Medical Science, 2-1-6 Kamikitazawa, Setagaya-ku, Tokyo 156-8506, Japan. yamagata-kn@igakuken.or.jp
Telephone: +81-3-68342358 Fax: +81-3-53163150
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## Abstract

Following injury, the axons of the mammalian central nervous system do not regenerate. Many studies have aimed at understanding the mechanisms that prevent axonal regeneration and at designing ways to overcome the obstacles preventing axonal regrowth. These studies have identified numerous proteins as promoters of axonal regeneration. In this minireviews, we focus on neuritin as a therapeutic candidate for promoting axonal regeneration. Neuritin was first identified as a neuronal-activity-inducible gene product in the rat brain. The overexpression of neuritin in neurons or the application of neuritin to neurons induces neuritogenesis, neurite arborization, and axonal elongation both *in vitro* and *in vivo*. These morphological changes are often observed during the first step of axonal regeneration. Indeed, neuritin expression increases during axonal regeneration in the peripheral nervous system (PNS). Conversely, in a mouse model of diabetes mellitus, neuritin expression decreases in the PNS, and this reduced expression may result in deficient axonal regeneration. Neuritin is induced in the hippocampal dentate gyrus after temporal lobe epilepsy or brain ischemia; however, in these conditions, neuritin induction may exacerbate brain dysfunction through mossy fiber sprouting. Together, these findings support the hypothesis that tightly controlled regulation of neuritin may be required for the treatment of each unique axonal pathology.

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**Key words:** Axonal regeneration; Neuronal development; Axonal injury; Neuritogenesis; Epilepsy

**Core tip:** Neuritin has been shown to be an activity-regulated protein in neurons. Its expression also increases after neuronal damage. Neuritin induces neuritogenesis, arborization, and axonal elongation. These functions may be beneficial for axonal regeneration after nerve injury. Here, we review neuritin as a therapeutic candidate for promoting axonal regeneration in the peripheral and central nervous systems. We also discuss the possible involvement of neuritin in mossy fiber sprouting after epileptic seizures or brain ischemia.

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## INTRODUCTION

After neuronal injury, axonal regeneration is not observed in the mammalian central nervous system (CNS). The regeneration of an axon itself is the only way to recover neuronal function after CNS injury. For decades, many investigators have tried to develop methods that promote axonal regeneration.

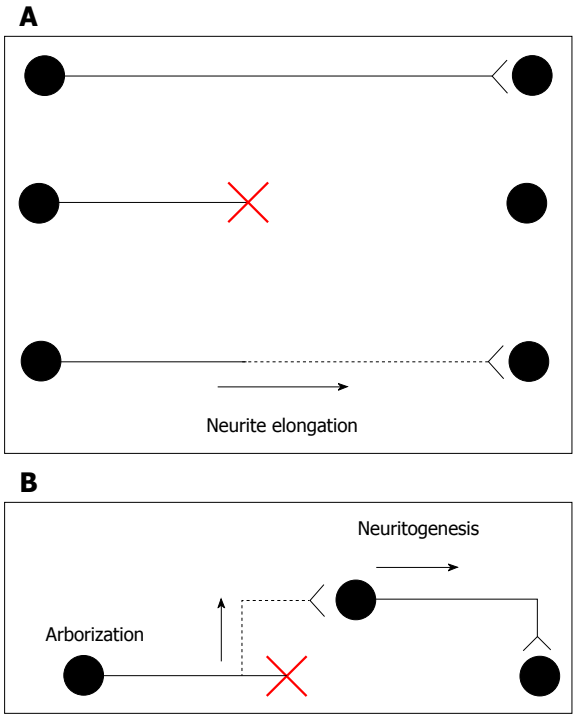


**Figure 1 Schematic illustration of axonal regeneration.** A: Top, intact axon; middle, transected or crushed axon; bottom, canonical axon regeneration. New growth occurs from the tip of the transected axon, and the regenerating axon reinnervates its normal target; B: Regenerating axon: the branch arises from the axon close to the injury site. The new axon branch connects to the surrounding neuron, which extends an axon to the original target. Figures are modified from Tuszynski *et al*[50].

In canonical axonal regeneration, new growth is expected to occur from the tip of a transected axon and reinnervate its normal target. Because CNS neurons maintain the intrinsic ability of axonal outgrowth[1], the promotion of neurite outgrowth can contribute to the regrowth of axons (Figure 1A). In addition to canonical regeneration, other processes can achieve the functional recovery of transected neurons. New axon branches could arise from the axonal region close to the injury site, and these branches could connect with other newborn neurons near the lesion. Alternatively, neural precursor cell grafts enable the production of newborn neurons. New neurons close to the injury site are expected to extend their axons to the target organs. In this model, enhancements of neuritogenesis and arborization help form new axons and branches, respectively (Figure 1B). A few transplant models have succeeded in promoting axonal functional recovery[2,3]. Thus, axonal elongation, branch formation, and neuritogenesis may be crucial for axonal regeneration.

In this minireviews, we focus on the protein neuritin, which is one candidate for the promotion of axonal regeneration. The expression level of neuritin is altered in CNS and peripheral nervous system (PNS) diseases. In addition, the overexpression or application of neuritin promotes neurite formation, branch formation, and neurite elongation in cultured neurons. These changes may promote axonal regeneration.

## NEURITIN: ITS CHARACTERISTICS AND DISTRIBUTION

Neuritin, which is also known as candidate plasticity gene 15 (CPG15), was first identified during a screen for neural-activity-regulated genes in the hippocampus[4]. Neuritin mRNA is predominantly expressed in the brain. Minor expression is observed in the lungs and liver[5]. Within the brain, the highest levels of expression are in the dentate gyrus of the hippocampus[5]. Expression of neuritin is increased by neural stimulation. Neuritin mRNA was increased both in the cerebral cortex by light[6], sensory experience[7], and exercise[8] and in the hippocampus by exercise[8] and electroconvulsive seizure[9]. Immunohistochemical analysis showed the expression of neuritin in the sciatic nerve[10] and facial motor neuron[11], indicating that neuritin is also expressed in the PNS.

An open reading frame of the neuritin sequence predicts a protein with 143 amino acids and a 27-amino-acid signal peptide. The 27 C-terminal amino acids contain a consensus cleavage signal that is found in glycosylphosphatidylinositol-anchored proteins. The predicted mature protein is a membrane-bound 12-kDa protein with no apparent phosphorylation or glycosylation site. Neuritin shows no significant homology with any known protein[5]. A portion of neuritin may be cleaved from the membrane and exists as a soluble form[12].

Neuritin protein is concentrated in the neuronal soma and is unevenly dispersed along neuritic projections in the rat brain[5]. Neuritin is expressed in puncta throughout cell bodies, dendrites, and axons, although its expression in axon terminals is particularly strong[13].

## NEURONAL FUNCTIONS OF NEURITIN

The neuronal functions of neuritin have been investigated primarily through studies involving the overexpression, gene silencing, and application of neuritin protein. These functions of neuritin can be categorized into the following three groups: neuritogenesis, neurite arborization, and neurite extension (Figure 2A).

### *Neuritogenesis*

After the administration of purified neuritin to cultured hippocampal and cortical neurons, neurons showed extensive dose-dependent neuritogenesis compared with control neurons[5,13]. In contrast, gene silencing of endogenous neuritin caused a robust reduction in nerve growth factor (NGF)-induced neurite formation[14]. Because NGF induces neuritin expression in dorsal root ganglion (DRG) neurons[10], NGF may promote neurite formation through neuritin expression; however, it remains unclear how NGF regulates neuritin expression. Furthermore, the application of a neutralizing antibody against neuritin to cultured cortical neurons significantly decreased the number of their primary dendrites and their total dendritic length[13]. These findings suggest that neuritin enhances neuritogenesis in developing neurons.

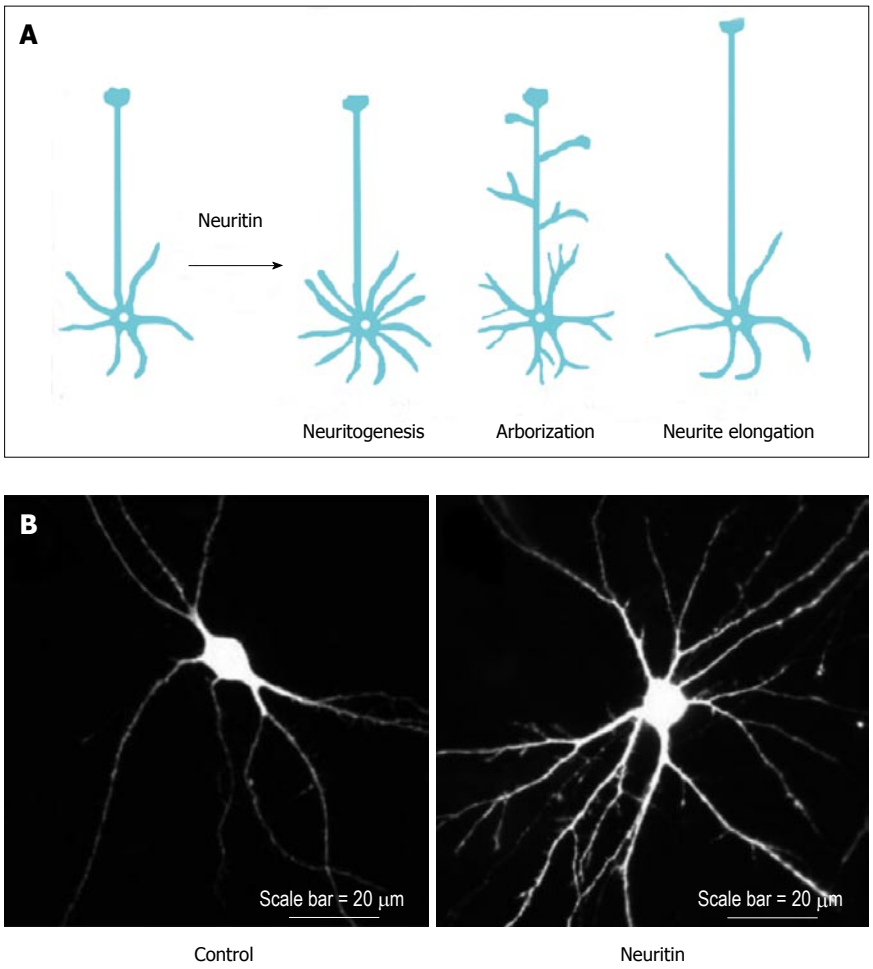


**Figure 2 Neuritin induces neurite arborization.** A: Neuritin regulates neuritogenesis, neurite arborization, and neurite extension; B: Hippocampal neurons were prepared from rat embryos that were transfected with green fluorescent protein (GFP) (left) or GFP and neuritin (right) after 5 d *in vitro*, and then they were immunostained after 12 d *in vitro*.

### *Neurite arborization*

Neuritin can promote branch formation in neuronal dendrites. The overexpression of neuritin increased dendritic arbors in the optic tectal neurons of *Xenopus* tadpoles[15]. Cultured rat hippocampal neurons overexpressing neuritin also exhibited significant increases in dendrite arborization (Figure 2B). Furthermore, neuritin targets axons to promote branch formation. The overexpression of neuritin changed the dynamic behavior of axonal branches. In the neuritin-expressing *Xenopus* motoneurons, the axonal branches grew significantly faster than those of control neurons owing to increased branch formation and decreased branch retraction[16].

### *Neurite extension*

In addition to neuritogenesis and branch formation, neuritin promotes the elongation of axons and dendrites. Dissociated cortical neurons plated on neuritin-coated dishes extended longer neurites than those plated on bovine serum albumin-coated dishes[5]. In addition, dissected hippocampal explants co-cultured with neuritin-expressing cells showed significantly longer neurites than those co-cultured with control cells[17]. Conversely, down regulation of neuritin expression completely abolished NGF-induced neurite outgrowth. The longest neurite of neuritin-reduced neurons treated with NGF was comparable to that of control neurons cultured without NGF[10]. Thus, there has been progress in our understanding of neuritin's functions, but by contrast, little is known about its mechanisms of action. A recent study has shed light on its signaling pathway (*e.g.*, the effects of neuritin on cerebellar granule neurons is attenuated by ERK, Akt, or mTOR inhibitors[18]); however, further work will be needed to dissect the mechanistic contributions of neuritin to different forms of neuronal function.

## POSSIBLE ROLES OF NEURITIN IN AXONAL REGENERATION

Neuritogenesis, axonal elongation, and axonal branching may be necessary for axonal regeneration. Accordingly, neuritin could contribute to axonal recovery. Several lines of evidence indicate that neuritin is involved in axonal regeneration in the PNS (Figure 3). In diabetes mellitus, axonal regeneration is nearly nonexistent in both experimental animal models[19,20] and clinical cases[21,22]. Streptozotocin (STZ) treatment causes experimental diabetic neuropathy in rats. Neuritin mRNA and protein have been shown to be significantly reduced in the DRG after STZ treatment[10]. The sections proximal to a ligature in the sciatic nerve exhibited a significant reduction in neuritin levels in diabetic rats compared with those

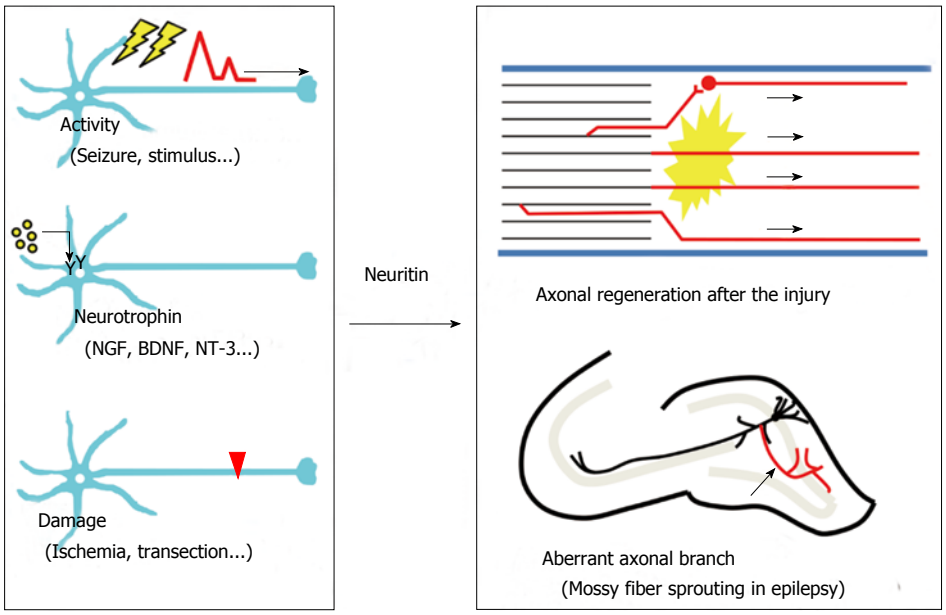


**Figure 3 Induction of neuritin and its pathophysiological functions.** Representative neuronal events that increase the expression of neuritin. Seizures, neurotrophins, and neuronal damage induce the expression of neuritin (left). The physiological roles of neuritin. Neuritin-induced changes might promote axonal regeneration after nerve injury, whereas the same morphological changes could exacerbate temporal lobe epilepsy (right). NGF: Nerve growth factor; BDNF: Brain-derived neurotrophic factor; NT-3: Neurotrophin-3.

in control rats, while distal sites did not[10]. These results suggest that the axonal transportation of neuritin to the ligature site may be reduced in diabetic rats, raising the possibility that experimental diabetic neuropathy could result from a deficiency in axonal regeneration caused by a decrease in neuritin expression.

What mechanism is involved in the reduction of neuritin in diabetic neuropathy? In cultured sensory neurons, NGF has been shown to increase neuritin mRNA and protein in a dose-dependent manner. NGF treatment reverses the decrease in neuritin levels in the DRGs of STZ-induced diabetic rats[10]. In addition, NGF levels are reduced in the diabetic PNS[23,24]. These results indicate that exogenous NGF may promote axonal regeneration in human diabetic patients *via* neuritin induction.

Androgen has been shown to induce axonal regeneration after the axotomy of various motor neurons[25]. After axotomy of the hamster facial nerve, exogenous androgen treatment lead to functional recovery by axonal regeneration[26]. Androgen treatment after axotomy of the facial nerve increased the expression of neuritin mRNA *in vivo*[11]. This androgen-dependent increase in neuritin mRNA was blocked by an antagonist of the androgen receptor[27]. Androgen-induced neuritin expression may participate in the axonal regeneration of other motor neuron injuries. Androgen may induce neuritin expression through a different mechanism than NGF signaling does. The neuritin promoter harbors seven putative androgen response elements[27], suggesting that the androgen receptor may directly control neuritin gene expression in motor neurons.

Neuritin mRNA has also been observed to increase after spinal cord injury. Fourteen days after injury by the weight-drop method, the amount of neuritin mRNA peaked near the injury site[28]. This finding may suggest that neuritin contributes to axonal regeneration in the CNS as well as in the PNS; however, future experiments are required to provide more definitive evidence. In addition, many questions remain regarding the molecular mechanisms by which neuritin expression is regulated under each pathological condition.

## INVOLVEMENT OF NEURITIN IN EPILEPTOGENESIS

Neuritin was first identified as a neural-activity-regulated gene product in the brain. In addition to the induction following spinal cord injury, neuritin expression is also drastically increased in the hippocampus in models of epilepsy and brain ischemia (Figure 3).

In temporal lobe epilepsy, granule cells in the hippocampal dentate gyrus show axonal sprouting and branching. This axonal change, which is called mossy fiber sprouting, is one of the hallmarks of an epileptic hippocampus observed in both experimental animal models[29-33] and clinical cases[34-38]. Mossy fiber sprouting leads to axonal projections into the inner molecular layer of the dentate gyrus and results in the formation of a closed recurrent circuit[29,39-42]. The formation of a new excitatory circuit within the dentate granule cells induces spontaneous bursting in the dentate gyrus under certain conditions[33,43-45]. The idea that mossy fiber sprouting is the main reason for acquired epileptogenesis is controversial; however, it is probable that increased axonal branching in the granule cells results in the formation of epileptogenic recurrent circuits in the hippocampus[46,47].

We have observed that neuritin promotes the arborization and elongation of mossy fibers in hippocampal slice cultures (unpublished data). This neuritin-induced mossy fiber sprouting may contribute to the formation of closed circuits between the granule cells (Figure 3). In this instance, an upregulation of neuritin may be harmful to brain function. Inhibition of neuritin function could be necessary for the suppression of epileptogenesis in temporal lobe epilepsy.

Transient middle cerebral artery occlusion (MCAO) leads to permanent damage of the brain that affects the lateral striatum and the lateral parietal, temporal and occipital cortex[48]. Two hours after MCAO treatment in rats, an induction of neuritin mRNA was observed in the ipsilateral dentate gyrus of the hippocampus, as well as in the ipsilateral cerebral cortex[49]. The increase in the hippocampus was robust and confined to this area 6 h after reperfusion[49]. In this model, the significance of neuritin induction in the cortex remains unclear; however, the upregulation of neuritin in dentate granule cells might indicate its involvement in the pathogenesis of postischemic epilepsy through mossy fiber sprouting (Figure 3).

Thus, aberrant neuritogenesis, axonal branch formation, and axonal elongation by neuritin could exacerbate neuronal dysfunction in the CNS. Tightly controlled regulation of neuritin expression may be required for the treatment of various CNS diseases.

## CONCLUSION

Neuritin is a small extracellular protein that is expressed in neurons, and its expression is upregulated by neuronal activity. The expression of neuritin increases in response to neuronal damage and neurotrophin treatment in the CNS and the PNS. Neuritin expression leads to morphological changes in neurons. The induction of neuritogenesis, the enhancement of neurite arborization, and the promotion of neurite extension are all elicited by neuritin. These morphological changes suggest that neuritin can contribute to axonal regeneration after axotomy. A variety of studies provide evidence that neuritin may contribute to axonal regeneration in PNS and CNS injuries. In contrast, neuritin induction in the hippocampus in response to epileptic seizures or brain ischemia may aggravate neuronal damage by enhancing mossy fiber sprouting. Thus, tightly controlled regulation of appropriate neuritin levels will be useful in the treatment of axonal pathology.

## REFERENCES


1 **David S**, Aguayo AJ. Axonal elongation into peripheral nervous system "bridges" after central nervous system injury in adult rats. *Science* 1981; **214**: 931-933 [PMID: 6171034]

2 **Ribotta MG**, Provencher J, Feraboli-Lohnherr D, Rossignol S, Privat A, Orsal D. Activation of locomotion in adult chronic spinal rats is achieved by transplantation of embryonic raphe cells reinnervating a precise lumbar level. *J Neurosci* 2000; **20**: 5144-5152 [PMID: 10864971]

3 **Pearse DD**, Marcillo AE, Oudega M, Lynch MP, Wood PM, Bunge MB. Transplantation of Schwann cells and olfactory ensheathing glia after spinal cord injury: does pretreatment with methylprednisolone and interleukin-10 enhance recovery? *J Neurotrauma* 2004; **21**: 1223-1239 [PMID: 15453992]

4 **Nedivi E**, Hevroni D, Naot D, Israeli D, Citri Y. Numerous candidate plasticity-related genes revealed by differential cDNA cloning. *Nature* 1993; **363**: 718-722 [PMID: 8515813]

5 **Naeve GS**, Ramakrishnan M, Kramer R, Hevroni D, Citri Y, Theill LE. Neuritin: a gene induced by neural activity and neurotrophins that promotes neuritogenesis. *Proc Natl Acad Sci USA* 1997; **94**: 2648-2653 [PMID: 9122250]

6 **Nedivi E**, Fieldust S, Theill LE, Hevron D. A set of genes expressed in response to light in the adult cerebral cortex and regulated during development. *Proc Natl Acad Sci USA* 1996; **93**: 2048-2053 [PMID: 8700883]

7 **Harwell C**, Burbach B, Svoboda K, Nedivi E. Regulation of cpg15 expression during single whisker experience in the barrel cortex of adult mice. *J Neurobiol* 2005; **65**: 85-96 [PMID: 16010668 DOI: 10.1002/neu.20176]

8 **Hunsberger JG**, Newton SS, Bennett AH, Duman CH, Russell DS, Salton SR, Duman RS. Antidepressant actions of the exercise-regulated gene VGF. *Nat Med* 2007; **13**: 1476-1482 [PMID: 18059283 DOI: 10.1038/nm1669]

9 **Newton SS**, Collier EF, Hunsberger J, Adams D, Terwilliger R, Selvanayagam E, Duman RS. Gene profile of electroconvulsive seizures: induction of neurotrophic and angiogenic factors. *J Neurosci* 2003; **23**: 10841-10851 [PMID: 14645477]

10 **Karamoysoyli E**, Burnand RC, Tomlinson DR, Gardiner NJ. Neuritin mediates nerve growth factor-induced axonal regeneration and is deficient in experimental diabetic neuropathy. *Diabetes* 2008; **57**: 181-189 [PMID: 17909094 DOI: 10.2337/db07-0895]

11 **Fargo KN**, Alexander TD, Tanzer L, Poletti A, Jones KJ. Androgen regulates neuritin mRNA levels in an in vivo model of steroid-enhanced peripheral nerve regeneration. *J Neurotrauma* 2008; **25**: 561-566 [PMID: 18419250 DOI: 10.1089/neu.2007.0466]

12 **Putz U**, Harwell C, Nedivi E. Soluble CPG15 expressed during early development rescues cortical progenitors from apoptosis. *Nat Neurosci* 2005; **8**: 322-331 [PMID: 15711540 DOI: 10.1038/nn1407]

13 **Sato H**, Fukutani Y, Yamamoto Y, Tatara E, Takemoto M, Shimamura K, Yamamoto N. Thalamus-derived molecules promote survival and dendritic growth of developing cortical neurons. *J Neurosci* 2012; **32**: 15388-15402 [PMID: 23115177 DOI: 10.1523/JNEUROSCI.0293-12.2012]

14 **Cappelletti G**, Galbiati M, Ronchi C, Maggioni MG, Onesto E, Poletti A. Neuritin (cpg15) enhances the differentiating effect of NGF on neuronal PC12 cells. *J Neurosci Res* 2007; **85**: 2702-2713 [PMID: 17335086 DOI: 10.1002/jnr.21235]

15 **Nedivi E**, Wu GY, Cline HT. Promotion of dendritic growth by CPG15, an activity-induced signaling molecule. *Science* 1998; **281**: 1863-1866 [PMID: 9743502 DOI: 10.1126/science.281.5384.1863]

16 **Javaherian A**, Cline HT. Coordinated motor neuron axon growth and neuromuscular synaptogenesis are promoted by CPG15 in vivo. *Neuron* 2005; **45**: 505-512 [PMID: 15721237 DOI: 10.1016/j.neuron.2004.12.051]

17 **Fujino T**, Wu Z, Lin WC, Phillips MA, Nedivi E. cpg15 and cpg15-2 constitute a family of activity-regulated ligands expressed differentially in the nervous system to promote neurite growth and neuronal survival. *J Comp Neurol* 2008; **507**: 1831-1845 [PMID: 18265009 DOI: 10.1002/cne.21649]

18 **Yao JJ**, Gao XF, Chow CW, Zhan XQ, Hu CL, Mei YA. Neuritin activates insulin receptor pathway to up-regulate Kv4.2-mediated transient outward K+ current in rat cerebellar granule neurons. *J Biol Chem* 2012; **287**: 41534-41545 [PMID: 23066017 DOI: 10.1074/jbc.M112.390260]

19 **Bisby MA**. Axonal transport of labeled protein and regeneration rate in nerves of streptozocin-diabetic rats. *Exp Neurol* 1980; **69**: 74-84 [PMID: 6156080 DOI: 10.1016/0014-4886(80)90144-2]

20 **Longo FM**, Powell HC, Lebeau J, Gerrero MR, Heckman H,

Myers RR. Delayed nerve regeneration in streptozotocin diabetic rats. *Muscle Nerve* 1986; **9**: 385-393 [PMID: 2941683]
21 **Sima AA**, Bril V, Nathaniel V, McEwen TA, Brown MB, Lattimer SA, Greene DA. Regeneration and repair of myelinated fibers in sural-nerve biopsy specimens from patients with diabetic neuropathy treated with sorbinil. *N Engl J Med* 1988; **319**: 548-555 [PMID: 3136331 DOI: 10.1056/NEJM198809013190905]
22 **Britland ST**, Young RJ, Sharma AK, Clarke BF. Association of painful and painless diabetic polyneuropathy with different patterns of nerve fiber degeneration and regeneration. *Diabetes* 1990; **39**: 898-908 [PMID: 2373262]
23 **Hellweg R**, Hartung HD. Endogenous levels of nerve growth factor (NGF) are altered in experimental diabetes mellitus: a possible role for NGF in the pathogenesis of diabetic neuropathy. *J Neurosci Res* 1990; **26**: 258-267 [PMID: 2142224]
24 **Anand P**, Terenghi G, Warner G, Kopelman P, Williams-Chestnut RE, Sinicropi DV. The role of endogenous nerve growth factor in human diabetic neuropathy. *Nat Med* 1996; **2**: 703-707 [PMID: 8640566]
25 **Fargo KN**, Foecking EM, Jones KJ, Sengelaub DR. Neuroprotective actions of androgens on motoneurons. *Front Neuroendocrinol* 2009; **30**: 130-141 [PMID: 19393684 DOI: 10.1016/j.yfrne.2009.04.005]
26 **Jones KJ**, Brown TJ, Damaser M. Neuroprotective effects of gonadal steroids on regenerating peripheral motoneurons. *Brain Res Brain Res Rev* 2001; **37**: 372-382 [PMID: 11744101 DOI: 10.1016/S0165-0173(01)00107-2]
27 **Marron TU**, Guerini V, Rusmini P, Sau D, Brevini TA, Martini L, Poletti A. Androgen-induced neurite outgrowth is mediated by neuritin in motor neurones. *J Neurochem* 2005; **92**: 10-20 [PMID: 15606892 DOI: 10.1111/j.1471-4159.2004.02836.x]
28 **Di Giovanni S**, Faden AI, Yakovlev A, Duke-Cohan JS, Finn T, Thouin M, Knoblach S, De Biase A, Bregman BS, Hoffman EP. Neuronal plasticity after spinal cord injury: identification of a gene cluster driving neurite outgrowth. *FASEB J* 2005; **19**: 153-154 [PMID: 15522907 DOI: 10.1096/fj.04-2694fje]
29 **Wenzel HJ**, Woolley CS, Robbins CA, Schwartzkroin PA. Kainic acid-induced mossy fiber sprouting and synapse formation in the dentate gyrus of rats. *Hippocampus* 2000; **10**: 244-260 [PMID: 10902894 DOI: 10.1002/1098-1063(2000)10:3<244: :AID-HIPO5>3.0.CO; 2-7]
30 **Kotti T**, Riekkinen PJ, Miettinen R. Characterization of target cells for aberrant mossy fiber collaterals in the dentate gyrus of epileptic rat. *Exp Neurol* 1997; **146**: 323-330 [PMID: 9270041 DOI: 10.1006/exnr.1997.6553]
31 **Buckmaster PS**, Dudek FE. Neuron loss, granule cell axon reorganization, and functional changes in the dentate gyrus of epileptic kainate-treated rats. *J Comp Neurol* 1997; **385**: 385-404 [PMID: 9300766 DOI: 10.1002/(SICI)1096-9861(19970901)385:3<385::AID-CNE4>3.0.CO;2-#]
32 **Okazaki MM**, Evenson DA, Nadler JV. Hippocampal mossy fiber sprouting and synapse formation after status epilepticus in rats: visualization after retrograde transport of biocytin. *J Comp Neurol* 1995; **352**: 515-534 [PMID: 7721998]
33 **Represa A**, Jorquera I, Le Gal La Salle G, Ben-Ari Y. Epilepsy induced collateral sprouting of hippocampal mossy fibers: does it induce the development of ectopic synapses with granule cell dendrites? *Hippocampus* 1993; **3**: 257-268 [PMID: 8353609 DOI: 10.1002/hipo.450030303]
34 **Kandratavicius L**, Hallak JE, Young LT, Assirati JA, Carlotti CG, Leite JP. Differential aberrant sprouting in temporal lobe epilepsy with psychiatric co-morbidities. *Psychiatry Res* 2012; **195**: 144-150 [PMID: 21741094 DOI: 10.1016/j.psychres.2011.06.005]
35 **Lynd-Balta E**, Pilcher WH, Joseph SA. AMPA receptor alterations precede mossy fiber sprouting in young children with temporal lobe epilepsy. *Neuroscience* 2004; **126**: 105-114 [PMID: 15145077 DOI: 10.1016/j.neuroscience.2004.03.004]
36 **El Bahh B**, Lespinet V, Lurton D, Coussemacq M, Le Gal La Salle G, Rougier A. Correlations between granule cell dispersion, mossy fiber sprouting, and hippocampal cell loss in temporal lobe epilepsy. *Epilepsia* 1999; **40**: 1393-1401 [PMID: 10528935]
37 **Zhang N**, Houser CR. Ultrastructural localization of dynorphin in the dentate gyrus in human temporal lobe epilepsy: a study of reorganized mossy fiber synapses. *J Comp Neurol* 1999; **405**: 472-490 [PMID: 10098940 DOI: 10.1002/(SICI)1096-9861(19990322)405:4<472: :AID-CNE3>3.0.CO; 2-P]
38 **Mathern GW**, Pretorius JK, Babb TL, Quinn B. Unilateral hippocampal mossy fiber sprouting and bilateral asymmetric neuron loss with episodic postictal psychosis. *J Neurosurg* 1995; **82**: 228-233 [PMID: 7815150]
39 **Buckmaster PS**, Zhang GF, Yamawaki R. Axon sprouting in a model of temporal lobe epilepsy creates a predominantly excitatory feedback circuit. *J Neurosci* 2002; **22**: 6650-6658 [PMID: 12151544]
40 **Cavazos JE**, Zhang P, Qazi R, Sutula TP. Ultrastructural features of sprouted mossy fiber synapses in kindled and kainic acid-treated rats. *J Comp Neurol* 2003; **458**: 272-292 [PMID: 12619081 DOI: 10.1002/cne.10581]
41 **Isokawa M**, Fried I. Extracellular slow negative transient in the dentate gyrus of human epileptic hippocampus in vitro. *Neuroscience* 1996; **72**: 31-37 [PMID: 8730703 DOI: 10.1016/0306-4522(95)00544-7]
42 **Patrylo PR**, Schweitzer JS, Dudek FE. Abnormal responses to perforant path stimulation in the dentate gyrus of slices from rats with kainate-induced epilepsy and mossy fiber reorganization. *Epilepsy Res* 1999; **36**: 31-42 [PMID: 10463848 DOI: 10.1016/S0920-1211(99)00022-4]
43 **Sutula T**, Cascino G, Cavazos J, Parada I, Ramirez L. Mossy fiber synaptic reorganization in the epileptic human temporal lobe. *Ann Neurol* 1989; **26**: 321-330 [PMID: 2508534]
44 **Wuarin JP**, Dudek FE. Electrographic seizures and new recurrent excitatory circuits in the dentate gyrus of hippocampal slices from kainate-treated epileptic rats. *J Neurosci* 1996; **16**: 4438-4448 [PMID: 8699254]
45 **Patrylo PR**, Dudek FE. Physiological unmasking of new glutamatergic pathways in the dentate gyrus of hippocampal slices from kainate-induced epileptic rats. *J Neurophysiol* 1998; **79**: 418-429 [PMID: 9425210]
46 **Hunt RF**, Scheff SW, Smith BN. Regionally localized recurrent excitation in the dentate gyrus of a cortical contusion model of posttraumatic epilepsy. *J Neurophysiol* 2010; **103**: 1490-1500 [PMID: 20089815 DOI: 10.1152/jn.00957.2009]
47 **Sutula TP**, Dudek FE. Unmasking recurrent excitation generated by mossy fiber sprouting in the epileptic dentate gyrus: an emergent property of a complex system. *Prog Brain Res* 2007; **163**: 541-563 [PMID: 17765737 DOI: 10.1016/S0079-6123(07)63029-5]
48 **Memezawa H**, Minamisawa H, Smith ML, Siesjö BK. Ischemic penumbra in a model of reversible middle cerebral artery occlusion in the rat. *Exp Brain Res* 1992; **89**: 67-78 [PMID: 1601103]
49 **Rickhag M**, Teilum M, Wieloch T. Rapid and long-term induction of effector immediate early genes (BDNF, Neuritin and Arc) in peri-infarct cortex and dentate gyrus after ischemic injury in rat brain. *Brain Res* 2007; **1151**: 203-210 [PMID: 17397810 DOI: 10.1016/j.brainres.2007.03.005]
50 **Tuszynski MH**, Steward O. Concepts and methods for the study of axonal regeneration in the CNS. *Neuron* 2012; **74**: 777-791 [PMID: 22681683 DOI: 10.1016/j.neuron.2012.05.006]

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MINIREVIEWS

# Antiplatelet strategy for acute ischemic stroke: A mini review

Zhong-He Zhou, Hui-Sheng Chen

Zhong-He Zhou, Hui-Sheng Chen, Department of Neurology, General Hospital of Shen-Yang Military Region, Shenyang 110840, Liaoning Province, China
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Correspondence to: Hui-Sheng Chen, MD, Professor, Department of Neurology, General Hospital of Shen-Yang Military Region, 83 Wenhua Road, Shenyang 110840, Liaoning Province, China. chszh@aliyun.com
Telephone: +86-24-28897511 Fax: +86-24-28856448
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**Core tip:** Patients with acute ischemic stroke have a high risk of deterioration in the 48-72 h after symptom onset. Although thrombolysis is an effective method, most patients are excluded due to the limit of strict indications. Early antiplatelets are recommended for most patients. However, the question remains unclear whether another effective and safe antiplatelet strategy for the treatment of acute ischemic stroke exists. Growing evidence shows that combination antiplatelets may be superior to mono antiplatelets in the treatment of acute ischemic stroke.

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## Abstract

Transient ischemic attacks and minor ischemic strokes have a high risk of an unstable clinical course in the initial 48-72 h after symptom onset. Early antiplatelet treatment is recommended to treat most patients with acute ischemic stroke because few patients can be treated with thrombolysis due to the limit of strict indications, such as a time window. Antiplatelets aim to prevent recurrence or deterioration of stroke. The guidelines recommend the use of aspirin in the acute stage based on two clinical trials. However, some patients still developed recurrence or deterioration of stroke despite timely aspirin administration. Thus, the question remains unclear whether another effective and safe antiplatelet strategy for the treatment of acute ischemic stroke exists. Growing evidence shows that combination antiplatelets may be superior to mono antiplatelets in the treatment of acute ischemic stroke.

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**Key words:** Antiplatelet; Acute ischemic stroke

## INTRODUCTION

In the acute stage of ischemic stroke, one of the most important treatment strategies aims to prevent recurrence or deterioration of stroke, because patients with transient ischemic attack (TIA) and minor ischemic stroke have a high risk of an unstable clinical course. Most of these unfavorable events occur in the initial 48-72 h after symptom onset and have obvious effects on the recovery of neurological functions in these patients[1-4]. It is well demonstrated that thrombolysis is an effective method to treat acute ischemic stroke. Due to the limit of strict indications, such as a time window, most patients are excluded for thrombolysis. Thus, early antiplatelet treatment is recommended to treat most patients with acute ischemic stroke in the guidelines[5,6]. In acute ischemic stroke, 2 mega trials established the efficacy of aspirin[7,8]; thus, the guidelines recommend the use of aspirin in the acute stage[5,6]. However, some patients develop recurrence or deterioration of stroke despite timely aspirin administration. Thus, the question remains unclear whether another effective and safe

antiplatelet strategy for the treatment of acute ischemic stroke exists.

In this article, we will briefly review the following topics: (1) the action mechanisms of different antiplatelet agents; (2) the evidence for antiplatelet therapy for acute ischemic stroke; and (3) the efficacy and safety of combination antiplatelet agents for acute ischemic stroke.

## ANTIPLATELET DRUGS AND MECHANISMS

Commonly used antiplatelet agents include aspirin, clopidogrel, cilostazol and dipyridamole. These agents act at different sites of the platelet aggregation pathway to inhibit platelet activation. Aspirin is a non-selective and irreversible cyclooxygenase (COX) inhibitor, which selectively acetylates the hydroxyl group of the COX enzyme, thus inhibiting conversion of arachidonate to prostaglandin G2/H2 and thromboxane A2. This leads to irreversible inhibition of platelet aggregation[9].

Clopidogrel is a thienopyridine derivative that prevents ADP-induced platelet activation and aggregation by irreversibly inhibiting the binding of adenosine 5-diphosphate to glycoprotein Ⅱb/Ⅲa, with the result of preventing the binding of fibrinogen to this receptor[10].

Cilostazol is an inhibitor of phosphodiesterase 3 (PDE3) which results in increased concentrations of intracellular cAMP[11]. Cilostazol inhibits primary and secondary platelet aggregation and high-shear stress platelet aggregation both *in vivo* and *in vitro*[12].

Dipyridamole is postulated to have multiple mechanisms of action. On one hand, its inhibition of platelet phosphodiesterase produces an increase in intraplatelet cyclic adenosine monophosphate level, resulting in the potentiation of the platelet inhibitory actions of prostacyclin. On the other hand, it also acts by directly releasing eicosanoid from vascular endothelium and inhibiting cellular uptake and metabolism of adenosine. This leads to the inhibition of platelet aggregation again[13].

## ANTIPLATELET THERAPY FOR ACUTE ISCHEMIC STROKE

A lot of studies have investigated the efficacy and safety of antiplatelet treatment in the secondary prevention of ischemic stroke[14]; however, only a few studies have investigated the efficacy and safety of antiplatelet treatment in acute ischemic stroke[7,8,15,16]. Of these studies, there are two powerful clinical trials: The Chinese Acute Stroke Trial (CAST)[8] and the International Stroke Trial (IST)[7]. In the CAST, 21,106 patients with acute ischemic stroke were enrolled in the clinical trial. Aspirin treatment (160 mg/d) was started within 48 h of the onset of a suspected acute ischemic stroke and continued in hospital for up to 4 wk. The results showed that aspirin treatment produced a significant reduction in mortality. Also, significantly fewer recurrent ischemic strokes occurred in the aspirin group compared with the placebo-allocated group, but slightly more hemorrhagic strokes. In the IST, 19435 patients with acute ischemic stroke within 48 h of symptom onset were enrolled. Antithrombotic therapy was started as soon as possible after stroke onset. The results showed that aspirin treatment significantly reduced death or recurrent ischemic strokes.

In addition to the two clinical trials, there are some completed clinical trials that investigated the antiplatelet strategy for acute ischemic stroke[15,16]. In the FASTER (the fast assessment of stroke and transient ischemic attack to prevent early recurrence) trial, 392 patients with TIA or minor stroke within 24 h of symptom onset were enrolled[15]. The trial was designed to investigate the effect of the combination of aspirin + clopidogrel versus aspirin monotherapy (with or without simvastatin) on the risk of recurrent stoke within 90 d after a TIA or minor stroke. The results showed the trend to lower recurrence or deterioration of stroke in the acute stage in the dual therapy group compared with mono antiplatelets. Another EARLY trial compared the safety and efficacy of aspirin + dipyridamole initiated within 24 h of stroke or TIA with that of aspirin + dipyridamole initiated after 7 d of aspirin monotherapy[16]. The results showed that early initiation of aspirin plus extended-release dipyridamole did not lead to differing disability at 90 d compared with late initiation, but the proportion of patients in the early initiation group who had no or mild disability on day 90 was higher than that in the late initiation group. There was also no significant difference in the composite adverse event endpoint between the two groups. These studies demonstrated the efficacy and safety of combined antiplatelet treatment in acute ischemic stroke. Furthermore, the proposal also got support from the CLAIR study[17]. The study aimed to investigate whether treatment with clopidogrel plus aspirin reduced the number of microembolic signals detected with transcranial doppler ultrasound compared with aspirin alone in patients with recent stroke. The results showed that combination therapy with clopidogrel and aspirin is more effective than aspirin alone in reducing microembolic signals in patients with predominantly intracranial symptomatic stenosis.

In other clinical trials aiming to investigate the secondary prevention for ischemic stroke, antiplatelet treatment was applied to some patients with acute ischemic stroke. For example, The ESPRIT Study compared aspirin with aspirin plus dipyridamole for secondary prevention after an ischemic stroke or transient ischemic attack[18]. The study recruited 96 patients during the acute stage of ischemic stroke and TIA. The Prevention Regimen for Effectively Avoiding Second Strokes (PRoFESS) trial is designed to compared extended-release dipyridamole plus aspirin *vs* clopidogrel in secondary stroke prevention[19]. The study recruited 1360 patients during the acute stage of ischemic stroke. These studies also demonstrated the efficacy and safety of combined antiplatelet treatment. Furthermore, a recent meta-analysis study show that dual antiplatelet therapy appears to

be safe and effective in reducing stroke recurrence and combined vascular events in patients with acute ischemic stroke or transient ischemic attack compared with monotherapy[20].

One powerful evidence comes from the Clopidogrel in High-risk Patients with Acute Nondisabling Cerebrovascular Events (CHANCE) study, which was just published in the *New England Journal of Medicine*[21]. The study showed that among patients with a TIA or minor stroke who can be treated within 24 h after the onset of symptoms, the combination of clopidogrel and aspirin is superior to aspirin alone for reducing the risk of stroke in the first 90 d and does not increase the risk of hemorrhage.

## BLEEDING EVENTS CAUSED BY ANTIPLATELETS

When considering using more intensive antiplatelet therapy, the balance between reducing recurrent stroke and increasing major bleeding events needs to be considered. Previous studies found that combination antiplatelet increased the bleeding events compared with mono antiplatelets. For example, the PRoFESS study found that aspirin plus extended-release dipyridamole produced more major hemorrhagic events than clopidogrel, including intracranial hemorrhage[22]. The MATCH study showed that life-threatening bleeding was higher in the group receiving aspirin and clopidogrel *vs* clopidogrel alone[23]. However, some studies found no difference between combination antiplatelets and mono antiplatelets, for example, the EARLY, FASTER and CLAIR trials[15-17]. More importantly, the recent CHANCE study also did not find more bleeding events with combined antiplatelets compared with mono antiplatelets[21]. The discrepancy could be due to the duration of antiplatelet treatment. Obviously, combination antiplatelets in the long-term trials such as PRoFESS and MATCH[22,23] produced more bleeding events. Thus, a time window of combination antiplatelets should exist at which risk outweighs benefit. Taken together, the evidence shows that combination antiplatelets in a short-term time window (maybe less than 3 wk) should be safe.

## CONCLUSION

As discussed above, although IST and CAST provided the most elegant evidence for aspirin in the acute stage of ischemic stroke, growing evidence, including CHANCE, points to the superiority of combination antiplatelets over mono antiplatelets. On one hand, due to their different mechanisms of action, it is postulated that combined antiplatelet agents may provide different degrees of vascular protection. On the other hand, the superiority of combination therapy compared to monotherapy may be due to the synergy effects of different antiplatelet mechanisms in reducing vascular events. Thus, combined antiplatelet therapy, such as aspirin and clopidogrel, should be encouraged in the treatment of acute ischemic stroke.

## REFERENCES


1 **Johnston SC**, Gress DR, Browner WS, Sidney S. Short-term prognosis after emergency department diagnosis of TIA. *JAMA* 2000; **284**: 2901-2906 [PMID: 11147987 DOI: 10.1001/jama.284.22.2901]

2 **Lovett JK**, Dennis MS, Sandercock PA, Bamford J, Warlow CP, Rothwell PM. Very early risk of stroke after a first transient ischemic attack. *Stroke* 2003; **34**: e138-e140 [PMID: 12855835 DOI: 10.1161/01.STR.0000080935.01264.91]

3 **Hill MD**, Yiannakoulias N, Jeerakathil T, Tu JV, Svenson LW, Schopflocher DP. The high risk of stroke immediately after transient ischemic attack: a population-based study. *Neurology* 2004; **62**: 2015-2020 [PMID: 15184607 DOI: 10.1212/01.WNL.0000129482.70315.2F]

4 **Coull AJ**, Lovett JK, Rothwell PM. Population based study of early risk of stroke after transient ischaemic attack or minor stroke: implications for public education and organisation of services. *BMJ* 2004; **328**: 326 [PMID: 14744823 DOI: 10.1136/bmj.37991.635266.44]

5 **Adams RJ**, Albers G, Alberts MJ, Benavente O, Furie K, Goldstein LB, Gorelick P, Halperin J, Harbaugh R, Johnston SC, Katzan I, Kelly-Hayes M, Kenton EJ, Marks M, Sacco RL, Schwamm LH. Update to the AHA/ASA recommendations for the prevention of stroke in patients with stroke and transient ischemic attack. *Stroke* 2008; **39**: 1647-1652 [PMID: 18322260 DOI: 10.1161/STROKEAHA.107.189063]

6 **European Stroke Organisation (ESO) Executive Committee**; ESO Writing Committee. Guidelines for management of ischaemic stroke and transient ischaemic attack 2008. *Cerebrovasc Dis* 2008; **25**: 457-507 [PMID: 18477843 DOI: 10.1159/000131083]

7 The International Stroke Trial (IST): a randomised trial of aspirin, subcutaneous heparin, both, or neither among 19435 patients with acute ischaemic stroke. International Stroke Trial Collaborative Group. *Lancet* 1997; **349**: 1569-1581 [PMID: 9174558 DOI: 10.1016/S0140-6736(97)04011-7]

8 CAST: randomised placebo-controlled trial of early aspirin use in 20,000 patients with acute ischaemic stroke. CAST (Chinese Acute Stroke Trial) Collaborative Group. *Lancet* 1997; **349**: 1641-1649 [PMID: 9186381 DOI: 10.1016/S0140-6736(97)04010-5]

9 **Vane JR**, Botting RM. The mechanism of action of aspirin. *Thromb Res* 2003; **110**: 255-258 [PMID: 14592543 DOI: 10.1016/S0049-3848(03)00379-7]

10 **Savi P**, Nurden P, Nurden AT, Levy-Toledano S, Herbert JM. Clopidogrel: a review of its mechanism of action. *Platelets* 1998; **9**: 251-255 [PMID: 16793712 DOI: 10.1080/09537109876799]

11 **Bradbury AW**. The role of cilostazol (Pletal) in the management of intermittent claudication. *Int J Clin Pract* 2003; **57**: 405-409 [PMID: 12846346]

12 **Reilly MP**, Mohler ER. Cilostazol: treatment of intermittent claudication. *Ann Pharmacother* 2001; **35**: 48-56 [PMID: 11197586 DOI: 10.1345/aph.19408]

13 **Harker LA**, Kadatz RA. Mechanism of action of dipyridamole. *Thromb Res Suppl* 1983; **4**: 39-46 [PMID: 6356465 DOI: 10.1016/0049-3848(83)90356-0]

14 **Tanne D**. Stroke: secondary stroke prevention--personalized antiplatelet therapy. *Nat Rev Neurol* 2012; **8**: 536-537 [PMID: 22926313 DOI: 10.1038/nrneurol.2012.180]

15 **Kennedy J**, Hill MD, Ryckborst KJ, Eliasziw M, Demchuk AM, Buchan AM. Fast assessment of stroke and transient ischaemic attack to prevent early recurrence (FASTER): a randomised controlled pilot trial. *Lancet Neurol* 2007; **6**: 961-969 [PMID: 17931979 DOI: 10.1016/S1474-4422(07)70250-8]

16 **Dengler R**, Diener HC, Schwartz A, Grond M, Schumacher H, Machnig T, Eschenfelder CC, Leonard J, Weissenborn K, Kastrup A, Haberl R. Early treatment with aspirin plus extended-release dipyridamole for transient ischaemic attack or ischaemic stroke within 24 h of symptom onset (EARLY trial): a randomised, open-label, blinded-endpoint

trial. *Lancet Neurol* 2010; **9**: 159-166 [PMID: 20060783 DOI: 10.1016/S1474-4422(09)70361-8]
17 **Wong KS**, Chen C, Fu J, Chang HM, Suwanwela NC, Huang YN, Han Z, Tan KS, Ratanakorn D, Chollate P, Zhao Y, Koh A, Hao Q, Markus HS. Clopidogrel plus aspirin versus aspirin alone for reducing embolisation in patients with acute symptomatic cerebral or carotid artery stenosis (CLAIR study): a randomised, open-label, blinded-endpoint trial. *Lancet Neurol* 2010; **9**: 489-497 [PMID: 20335070 DOI: 10.1016/S1474-4422(10)70060-0]
18 **Halkes PH**, van Gijn J, Kappelle LJ, Koudstaal PJ, Algra A. Aspirin plus dipyridamole versus aspirin alone after cerebral ischaemia of arterial origin (ESPRIT): randomised controlled trial. *Lancet* 2006; **367**: 1665-1673 [PMID: 16714187 DOI: 10.1016/S0140-6736(06)68734-5]
19 **Bath PM**, Cotton D, Martin RH, Palesch Y, Yusuf S, Sacco R, Diener HC, Estol C, Roberts R. Effect of combined aspirin and extended-release dipyridamole versus clopidogrel on functional outcome and recurrence in acute, mild ischemic stroke: PRoFESS subgroup analysis. *Stroke* 2010; **41**: 732-738 [PMID: 20181679 DOI: 10.1161/STROKEAHA.109.564906]
20 **Geeganage CM**, Diener HC, Algra A, Chen C, Topol EJ, Dengler R, Markus HS, Bath MW, Bath PM. Dual or mono antiplatelet therapy for patients with acute ischemic stroke or transient ischemic attack: systematic review and meta-analysis of randomized controlled trials. *Stroke* 2012; **43**: 1058-1066 [PMID: 22282894 DOI: 10.1161/STROKEAHA.111.637686]
21 **Wang Y**, Wang Y, Zhao X, Liu L, Wang D, Wang C, Wang C, Li H, Meng X, Cui L, Jia J, Dong Q, Xu A, Zeng J, Li Y, Wang Z, Xia H, Johnston SC. Clopidogrel with aspirin in acute minor stroke or transient ischemic attack. *N Engl J Med* 2013; **369**: 11-19 [PMID: 23803136 DOI: 10.1056/NEJMoa1215340]
22 **Sacco RL**, Diener HC, Yusuf S, Cotton D, Ounpuu S, Lawton WA, Palesch Y, Martin RH, Albers GW, Bath P, Bornstein N, Chan BP, Chen ST, Cunha L, Dahlöf B, De Keyser J, Donnan GA, Estol C, Gorelick P, Gu V, Hermansson K, Hilbrich L, Kaste M, Lu C, Machnig T, Pais P, Roberts R, Skvortsova V, Teal P, Toni D, Vandermaelen C, Voigt T, Weber M, Yoon BW. Aspirin and extended-release dipyridamole versus clopidogrel for recurrent stroke. *N Engl J Med* 2008; **359**: 1238-1251 [PMID: 18753638 DOI: 10.1056/NEJMoa0805002]
23 **Diener HC**, Bogousslavsky J, Brass LM, Cimminiello C, Csiba L, Kaste M, Leys D, Matias-Guiu J, Rupprecht HJ. Aspirin and clopidogrel compared with clopidogrel alone after recent ischaemic stroke or transient ischaemic attack in high-risk patients (MATCH): randomised, double-blind, placebo-controlled trial. *Lancet* 2004; **364**: 331-337 [PMID: 15276392 DOI: 10.1016/S0140-6736(04)16721-4]

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CASE REPORT

# Atypical neurological symptoms associated with CGG expansions of the *FMR1* gene

Esteban Peña, Marcos Llanero

Esteban Peña, Marcos Llanero, Department of Neurology, Hospital Sanitas La Moraleja, 28050 Madrid, Spain
Author contributions: Peña E contributed to writing and reviewing the manuscript; Llanero M contributed to neuropsychological assessment and reviewing the manuscript.
Correspondence to: Esteban Peña, MD, Department of Neurology, Hospital Sanitas La Moraleja, Avenida Francisco Pi y Margall n° 81, 28050 Madrid, Spain. epenal.pex@sanitas.es
Telephone: +34-917-679100 Fax: +34-917-679346
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## Abstract

More than 40 CGG expansions in the 5' noncoding region of the fragile X mental retardation 1 (*FMR1*) gene of the X chromosome give rise to several distinct clinical phenotypes, depending on the size of the expansion. First, more than 200 CGG expansions (full mutation) cause an inherited mental retardation called fragile X syndrome. Second, CGG expansions between 55 and 199 (premutation) cause a disorder called fragile X-associated tremor/ataxia syndrome (FXTAS) which typically includes intention tremor, ataxia and specific magnetic resonance imaging (MRI) findings. Indeed, it could develop parkinsonism although it usually shows features of postsynaptic parkinsonism. Finally, CGG expansions between 41 and 54 CGG (gray zone) are not consider normal but rarely develops abnormal neurological conditions. In this sense, the aim of this study is to report two atypical cases associated with CGG expansions of the *FMR1* gene. First, a FMR1 premutation alleles carrier with an unusual phenotype, such as a presynaptic parkinsonism indistinguishable from Parkinson disease (PD) and a FMR1 gray zone alleles carrier presented with neurological features, namely hand tremor, parkinsonism and ataxia, usually described in FXTAS, as well as orthostatic tremor. We conclude that, on the one hand, FMR1 premutation alleles might cause two phenotypes of parkinsonism, such as a presynaptic phenotype, indistinguishable from PD, and a postsynaptic phenotype, associated with clinical features of FXTAS. On the other hand, although FMR1 gray zone alleles carriers were believed to have no abnormal neurological conditions, our study supports that they could develop FXTAS and other neurological disorders such as orthostatic tremor which has not been reported before associated with the *FMR1* gene.

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**Key words:** Fragile X-associated tremor/ataxia syndrome; Fragile X mental retardation; Gray zone; Parkinsonism; Orthostatic tremor

**Core tip:** In this study we report two atypical cases associated with CGG expansions of the fragile X mental retardation 1 (*FMR1*) gene. First, a FMR1 premutation alleles carrier presented with a parkinsonism indistinguishable from Parkinson disease. Second, a FMR1 gray zone alleles carrier presented with orthostatic tremor and neurological features associated with the fragile X-associated tremor/ataxia syndrome, such as hand tremor, parkinsonism and ataxia.

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## INTRODUCTION

More than 40 CGG expansions in the 5' noncoding region of the fragile X mental retardation 1 (*FMR1*) gene

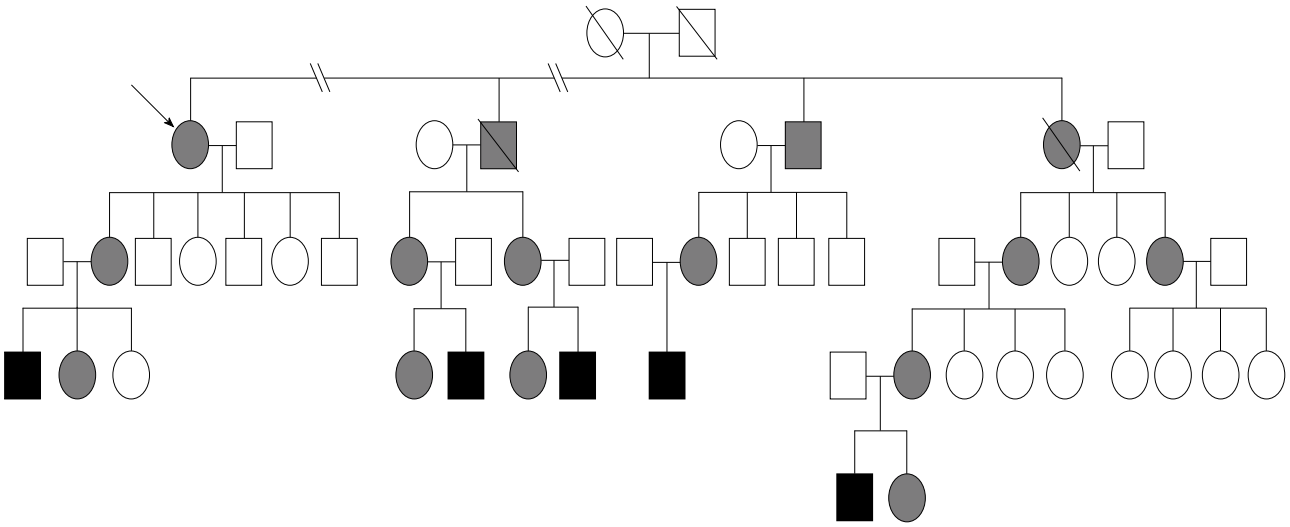

**Figure 1 Pedigree structure of the patient 1 (arrow), they were 12 brothers and sisters, only those relevant are shown.** Fragile X mental retardation (FMR1) CGG expanded alleles carriers are denoted as a grey filled symbol, they did not show any neurological disorder. Black filled symbols denote FMR1 CGG full mutation carriers affected by fragile X syndrome with mental retardation. Crossed symbol indicates deceased family members. Circle indicates females and square males.

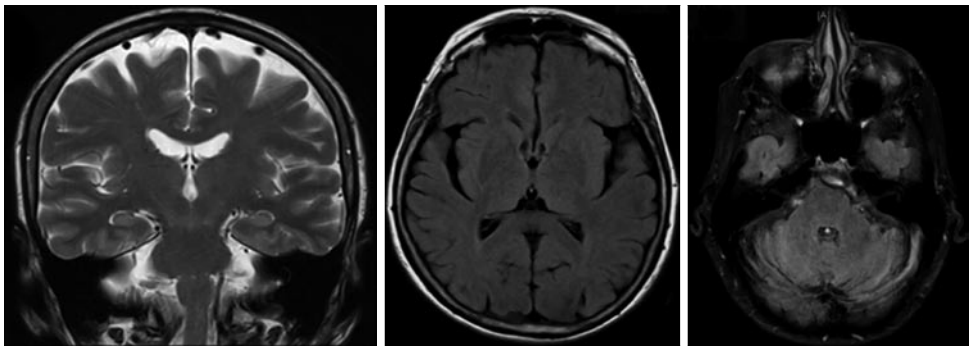


**Figure 2 T2, TIR and FLAIR magnetic resonance imaging of the patient 1, it does not show abnormal findings.** FLAIR: Fluid attenuated inversion recovery.

of the X chromosome give rise to several distinct clinical phenotypes, depending on the size of the expansion. First, fragile X syndrome is an inherited mental retardation caused by more than 200 CGG expansions in the *FMR1* gene (full mutation). Second, fragile X-associated tremor/ataxia syndrome (FXTAS) is a disorder described in premutation alleles carriers (55-199 CGG expansions) of the *FMR1* gene. Typically, it includes intention tremor, ataxia and specific magnetic resonance imaging (MRI) findings, although the spectrum of neurological disorders associated with the FXTAS is increasing[1-3]. Finally, an interval of CGG expansions between 41 and 54 is known as gray zone which rarely develops abnormal neurological conditions. In this sense, the aim of this study is to report two atypical cases associated with CGG expansions of the *FMR1* gene. First, a FMR1 premutation alleles carrier with an unusual phenotype, such as a parkinsonism indistinguishable from Parkinson disease (PD) and a FMR1 gray zone alleles carrier presented with neurological features, namely hand tremor, parkinsonism, ataxia and orthostatic tremor.

## CASE REPORT

### *Case 1*

A 71-year-old woman presented with a 4-year history of progressive neurological disorder with tremor, bradykinesia and gait disorder. She was FMR1 alleles carrier (expansion of 82 CGG) with family history of fragile X syndrome (Figure 1). On examination, she had severe rest and postural tremor in her right hand and slight postural tremor in her left hand, severe bradykinesia and rigidity more marked on her right side, mild lost of postural stability and freezing. There was neither intention tremor nor ataxia. Indeed, patient did not show either autonomic dysfunction or Babinski sign or gaze palsy. Finally, She had significant improvement with 520 mg of levodopa (Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale I 8 Ⅱ 17 Ⅲ ON 42 OFF 60 Ⅳ 0). These clinical findings fulfilled the United Kingdom PD Society Brain Bank clinical diagnostic criteria for idiopathic PD[4].

Blood tests and cranial MRI were normal (Figure 2).

**Table 1 Neuropsychological assessment of the patient 1**

| Test | Subtest | Percentile |
|---|---|---|
| Orientation | | Normal |
| Attention and executive functions | | |
| D2 test of attention | Processing elements | p45 |
| | Omissions | p25 |
| | Concentration | p40 |
| Digit span (WMS-III) | Forward | p50 |
| | Backward | p50 |
| Verbal fluency | Semantic (animals) | p75 |
| | Semantic (names) | p90 |
| | Phonological (FAS) | p30 |
| Trail making test | A | p50 |
| | B | p20 |
| Stroop | | p50 |
| Hanoi tower | 3 disk | Normal |
| | 4 disk | Not solved[1] |
| Similarities (WAIS) | | p50 |
| Wisconsin card sorting test | Categories completed | 4 (P > 16) |
| | Failure to maintain set | p51 |
| | Errors | p30 |
| | Perseverative responses | p30 |
| | Perseverative errors | p30 |
| | Conceptual level responses | p20 |
| BADS | Rule shift cards | p51 |
| | DEX | Score 20 |
| Episodic memory | | |
| Logical memory Ⅰ | Recall unit (Story A + B) | p20 |
| (WMS-III) | Recall thematic (Story A+ B) | p20 |
| Logical memory Ⅱ | Recall unit (Story A + B) | p30 |
| (WMS-III) | Recall thematic (Story A + B) | p30 |
| Word list Ⅰ (WMS-III) | Recall total score | p50 |
| | Short-delay recall | p85 |
| Word list Ⅱ (WMS-III) | Recall | p70 |
| | Recognition | p85 |
| Rey-osterrieth complex figure test | Recall | p50 |
| Naming | | |
| Boston naming test | | p50 |
| Praxis and visuo-constructive ability | | |
| Rey-osterrieth complex figure test | Copy | p75 |
| Gesture imitation | | Normal |
| Complex sequencing | | Normal |

[1]It shows slight deficits in speed information processing, in planning as well as in attention to designated targets and to inhibit responses. FAS: Foreign Agricultural Service; WAIS-III: Wechsler Adult Intelligence Scale-III; WAIS: Wechsler Adult Intelligence Scale; BADS: Behavioural Assessment of the Dysexecutive Syndrome.

DAT SCAN showed an asymmetric decrease of the striatum tracer uptake more marked on the left side (Figure 3). Neuropsychological assessment did not show cognitive decline. However, it showed slight subcortical deficits (Table 1).

### *Case 2*

A 76-year-old man, FMR1 gray-zone alleles carrier (expansion of 51 CGG) presented with a 3-year history of hand tremor and severe gait disorder. On examination he had mild postural tremor in both hands, mild rigidity more marked in the left side, lost of postural stability, severe tremor in a standing position, ataxia and freezing.

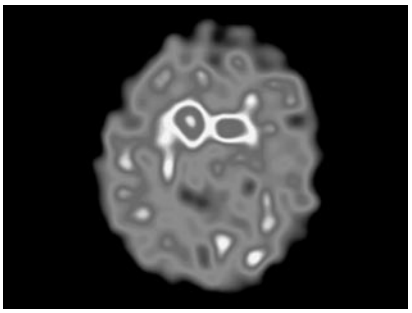

**Figure 3 [$^{123}$I]FP-CIT DAT SCAN of the patient 1, it shows an asymmetric decrease of the striatum tracer uptake more marked on the left side.**

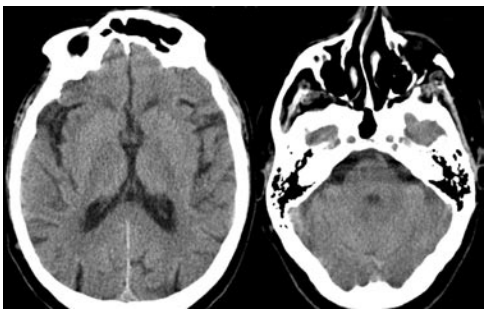

**Figure 4 Cranial computed tomography of the patient 2, it does not show relevant findings.**

He improved neither with 609 mg of levodopa nor with 1.5 mg of clonazepam. There were not cognitive decline.

Blood tests were normal. Cranial computed tomography and DAT SCAN were also normal (Figures 4 and 5). Electromyography corroborated high-frequency (14 Hz) tremor in a standing position in both legs which confirmed the diagnosis of orthostatic tremor.

## DISCUSSION

Parkinsonism is often related to FXTAS, although most patients do not meet clinical criteria for PD[1,2]. Indeed, in parkinsonism related to FXTAS, response to levodopa is often poor[5,6], DAT SCAN is usually normal[5] and Single Photon Emission Computed Tomography with iodobenzamide has shown abnormal tracer uptake[6]. It leads to conclude that parkinsonism in FXTAS is developed by dysfunction beyond the presynaptic nigroestriatal level[5]. However, our first case, a parkinsonism with clinical features indistinguishable from PD, as similar cases previously reported[2], without intention tremor or ataxia and with both good response to levodopa and abnormal DAT SCAN suggests damage restricted to the presynaptic level. Thus, although the relationship between these "presynaptic parkinsonisms" and the FMR1 premutation could be casual, it is necessary to consider that the FMR1 premutation might cause two phenotypes of parkinsonism, such as a presynaptic phenotype, indistinguishable from PD, and a postsynaptic phenotype, associated with clinical features of FXTAS. In fact, neuropathological features related to the *FMR1* gene without Lewy bodies

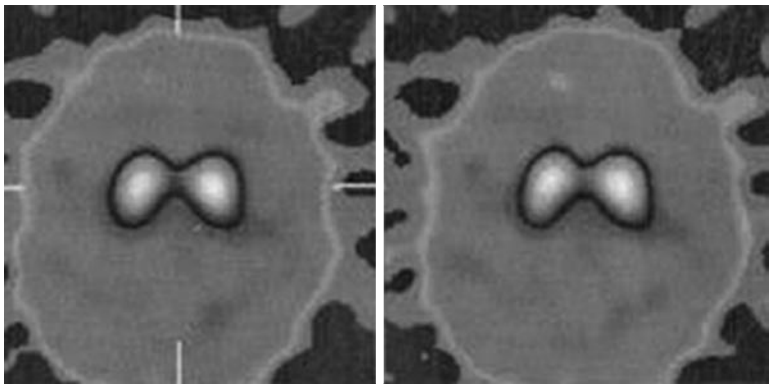

**Figure 5 [$^{123}$I]FP-CIT DAT SCAN of the patient 2, it shows a symmetric and normal tracer uptake.**

were found in a FMR1 premutation alleles carrier with clinical features of presynaptic parkinsonism[7] which supported the relationship between that presynaptic parkinsonism and the *FMR1* gene as well as ruled out a concomitant PD. How to explain it remains unclear, it could reflect either different ranges of severity or different stages of the same process or the coexistence of the FMR1 premutation with others genes associated with PD[8]. Thus, we suggest that FMR1 premutation should be considered in the differential diagnosis of PD, almost in those patients with family history of disorders related to the *FMR1* gene.

FMR1 gray zone alleles carriers were believed to have no abnormal neurological conditions. However, a recent report has suggested a relationship between FMR1 gray zone alleles carriers and FXTAS[3]. In this sense, our second case, presented with usual clinical features of FXTAS, such as parkinsonism, hand tremor and ataxia, supports the fact that FMR1 gray zone alleles carriers could develop FXTAS. Indeed, orthostatic tremor has not been described before in association with the *FMR1* gene. Thus, the presence of orthostatic tremor in our second patient expands the clinical spectrum of neurological disorders associated with the *FMR1* gene. Future research should validate findings on a larger group of patients.

## ACKNOWLEDGMENTS

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## REFERENCES

1 **Jacquemont S**, Hagerman RJ, Leehey M, Grigsby J, Zhang L, Brunberg JA, Greco C, Des Portes V, Jardini T, Levine R, Berry-Kravis E, Brown WT, Schaeffer S, Kissel J, Tassone F, Hagerman PJ. Fragile X premutation tremor/ataxia syndrome: molecular, clinical, and neuroimaging correlates. *Am J Hum Genet* 2003; **72**: 869-878 [PMID: 12638084 DOI: 10.1086/374321]

2 **Hall DA**, Howard K, Hagerman R, Leehey MA. Parkinsonism in FMR1 premutation carriers may be indistinguishable from Parkinson disease. *Parkinsonism Relat Disord* 2009; **15**: 156-159 [PMID: 18565783 DOI: 10.1016/j.parkreldis.2008.04.037]

3 **Hall D**, Tassone F, Klepitskaya O, Leehey M. Fragile X-associated tremor ataxia syndrome in FMR1 gray zone allele carriers. *Mov Disord* 2012; **27**: 296-300 [PMID: 22161987 DOI: 10.1002/mds.24021]

4 **Hughes AJ**, Daniel SE, Kilford L, Lees AJ. Accuracy of clinical diagnosis of idiopathic Parkinson's disease: a clinico-pathological study of 100 cases. *J Neurol Neurosurg Psychiatry* 1992; **55**: 181-184 [PMID: 1564476 DOI: 10.1136/jnnp.55.3.181]

5 **Ceravolo R**, Antonini A, Volterrani D, Rossi C, Goldwurm S, Di Maria E, Kiferle L, Bonuccelli U, Murri L. Dopamine transporter imaging study in parkinsonism occurring in fragile X premutation carriers. *Neurology* 2005; **65**: 1971-1973 [PMID: 16380622 DOI: 10.1212/01.wnl.0000188821.51055.52]

6 **Scaglione C**, Ginestroni A, Vella A, Dotti MT, Nave RD, Rizzo G, De Cristofaro MT, De Stefano N, Piacentini S, Martinelli P, Mascalchi M. MRI and SPECT of midbrain and striatal degeneration in fragile X-associated tremor/ataxia syndrome. *J Neurol* 2008; **255**: 144-146 [PMID: 18080849 DOI: 10.1007/s00417-007-0711-8]

7 **Louis E**, Moskowitz C, Friez M, Amaya M, Vonsattel JP. Parkinsonism, dysautonomia, and intranuclear inclusions in a fragile X carrier: a clinical-pathological study. *Mov Disord* 2006; **21**: 420-425 [PMID: 16250026 DOI: 10.1002/mds.20753]

8 **Hedrich K**, Pramstaller PP, Stübke K, Hiller A, Kabakci K, Purmann S, Kasten M, Scaglione C, Schwinger E, Volkmann J, Kostic V, Vieregge P, Martinelli P, Abbruzzese G, Klein C, Zühlke C. Premutations in the FMR1 gene as a modifying factor in Parkin-associated Parkinson's disease? *Mov Disord* 2005; **20**: 1060-1062 [PMID: 15929093 DOI: 10.1002/mds.20512]

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*In press*

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

*Organization as author*

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

*Both personal authors and an organization as author*

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

*No author given*

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

*Volume with supplement*

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

*Issue with no volume*

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

*No volume or issue*

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

**Books**

*Personal author(s)*

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

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11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

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12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

*Conference proceedings*

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

*Conference paper*

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

**Electronic journal** (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

**Patent** (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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