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SCIENTIFIC COMMENTARIES

When all is lost. . .a severe myopathy with hypotonia from sodium channel mutations

This scientific commentary refers to "Loss-of-function mutations in *SCN4A* cause severe foetal hypokinesia or 'classical' congenital myopathy", by Zaharieva *et al.* (doi:10.1093/brain/awv352).

The sodium channel is the work horse for the generation and propagation of action potentials in nerve, muscle and heart. The exquisite tuning of excitability in these cells to meet the body's needs for impulse propagation, initiation of contraction, triggering of neurotransmitter release, or pacemaking is achieved by a highly orchestrated regulation of ion channel gene expression. In the case of voltage-gated sodium channels, the human genome contains nine separate genes encoding pore-forming α-subunits ($Na_v1.1$–$Na_v1.9$) (George, 2005). In skeletal muscle the $Na_v1.4$ isoform predominates and carries $>90\%$ of the total sodium current. Mutations of the *SCN4A* gene encoding $Na_v1.4$ are an established cause of several muscle disorders presenting with myotonia, familial periodic paralysis, and in very rare instances, congenital myasthenia (Cannon, 2015). In this issue of *Brain*, Zaharieva and colleagues identify an additional phenotype with severe congenital myopathy and neonatal hypotonia when both *SCN4A* alleles harbour recessive loss-of-function mutations (Zaharieva *et al.*, 2016).

Over 60 mutations of *SCN4A* have been identified in patients with paroxysmal disorders of skeletal muscle that present with myotonia or periodic paralysis (Lehmann-Horn and Jurkat-Rott, 1999). All are missense mutations in which a single amino acid is replaced, producing a 'gain-of-function' defect with a pathological excess of inward sodium current (Cannon, 2015). The gain-of-function most often occurs from impaired inactivation of mutant channels (incomplete, too slow, or shifted in voltage-dependence to more positive potentials), but may also arise from enhanced activation (lower voltage threshold). The aberrant sodium current promotes fibre depolarization and thereby creates susceptibility to prolonged busts of after-discharges that cause the persistent after-contraction of myotonia. With more severe gain-of-function defects, the resting potential may become stably depolarized, which renders the fibre refractory from firing impulses and causes the flaccid weakness of periodic paralysis. Because these channel mutations cause gain-of-function defects, their inheritance is dominant as seen in the sodium channel myotonias (potassium-aggravated myotonia, myotonia fluctuans, myotonia permanens), paramyotonia congenita, or hyperkalemic periodic paralysis. A variation on the gain-of-function theme occurs for *SCN4A* mutations associated with hypokalemic periodic paralysis. In this case, missense mutations in the voltage-sensor domain produce an anomalous ion-conducting pathway that results in 'leaky' mutant $Na_v1.4$ channels (Struyk and Cannon, 2007). This leak increases the likelihood of resting potential depolarization and loss of excitability at low–normal levels of extracellular potassium.

Loss-of-function mutations in *SCN4A* are much less common than gain-of-function changes and are associated with more severe muscle phenotypes. The first *SCN4A* loss-of-function mutation was identified in a patient with life-long respiratory and bulbar weakness, fatigability with modest exertion, and a decrement in motor responses during repetitive nerve stimulation at 10 Hz or greater (Tsujino *et al.*, 2003). An evaluation for congenital myasthenia surprisingly showed normal quantal release, acetylcholine receptor kinetics, and endplate structure, but the evoked endplate potential to -40 mV failed to elicit a muscle action potential. This constellation of findings implied a possible defect of $Na_v1.4$ and a missense mutation was identified. Functional testing showed a profound -30 mV shift in the voltage dependence of inactivation, which is predicted to render nearly 90% of the mutant channels inactive at the resting potential. A second case of myasthenia in association with an *SCN4A* mutation was reported for a recessively inherited missense mutation that also produced a loss-of-function with a leftward voltage shift of inactivation and 10-fold slower recovery from inactivation (Arnold *et al.*, 2015). The heterozygous parents and siblings were asymptomatic.

Glossary

Compound heterozygote: An individual for whom a recessive trait is expressed because of the inheritance of two different mutant alleles at a particular gene locus, one on each chromosome.

Hypomorphic allele: A type of mutation in which the activity of the gene product or the level of gene expression is reduced, but is not completely lost as occurs with a null allele.

Zaharieva and co-workers now further extend the clinical phenotypes associated with severe loss-of-function changes for $Na_v1.4$ in a cohort of patients with congenital myopathy and neonatal hypotonia (Zaharieva *et al.*, 2016). The associations were revealed by a comprehensive screen using whole exome sequencing, rather than the presence of a clinical or neurophysiological trait that would implicate the sodium channel. Homozygous or compound heterozygous mutations of *SCN4A* were identified in 11 individuals from six unrelated congenital myopathy families in an international consortium. For the first time, null alleles of *SCN4A* were identified wherein a nonsense, frameshift or splice site mutation destroyed the coding potential for a functional $Na_v1.4$ channel. Moreover, no current was detectable in expression studies for three of six missense mutations. Inheritance of two functionally null $Na_v1.4$ alleles was lethal in the third trimester or within minutes of birth. The cardiac sodium channel, $Na_v1.5$, is expressed in embryonic skeletal muscle and then decreases postnatally (Yang *et al.*, 1991), but was not able to support survival in a complete $Na_v1.4$ null. Four of the 11 individuals survived (now aged 2.5 to 35 years), all of whom had one null allele while the other was hypomorphic with reduced sodium current densities of 20–80% of normal levels. One mutant allele with a distal carboxyl terminal frameshift mutation had normal-appearing sodium currents when expressed in fibroblasts, but the missense changes to the terminal amino acids eliminate a conserved sequence (PDZ protein interaction domain) that is predicted to disrupt localization of the channel in the plasma membrane. The four survivors had marked congenital hypotonia, neonatal respiratory and swallowing difficulties, weakness most pronounced in face and neck muscles, and skeletal abnormalities of the spine and palate. All improved with age and acquired independent ambulation. Three of four experienced rapid onset fatigue with walking, and one of two tested had a compound muscle action potential (CMAP) decrement with repetitive nerve stimulation at 10 Hz, but myasthenia was not considered to be a feature of the syndrome. One had episodic weakness after exercise or with fever, but CMAP amplitudes were normal in short- and long-exercise tests, and periodic paralysis was not a feature either. Surprisingly, relatives with one null allele were asymptomatic and had no muscle abnormality detected on clinical examination.

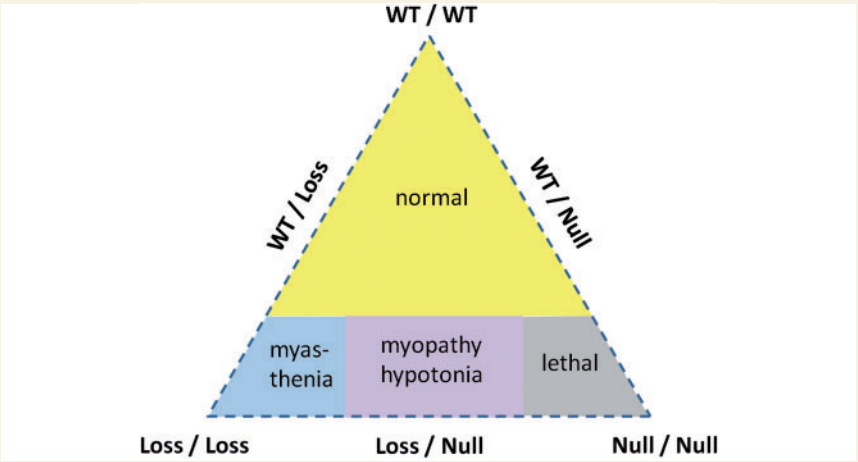

Figure 1 Schematic representation of genotype-phenotype associations for loss-of-function mutations of $Na_v1.4$. WT = wild-type.

A synthesis of the genotype–phenotype relation for loss-of-function defects of $Na_v1.4$ is depicted in Fig. 1. The vertices of the triangle represent homozygous states for the three possible alleles: wild-type, hypomorphic loss of function (Loss), and complete absence of function (Null). The possible heterozygous combinations are represented as the midpoint on each side of the triangle. A single copy of a mutant loss-of-function allele (partial or complete) is well tolerated, as shown by the large domain for a normal phenotype (yellow). The presence of two mutant alleles is always symptomatic and increases in severity from Loss/Loss producing myasthenia, Loss/Null causing hypotonia with congenital myopathy, and Null/Null being lethal. The human condition, of course, is more complex than this simplified scheme and so boundaries will be blurred and domains will overlap on the phenotype map. For example, severe neonatal hypotonia has also been reported with a dominantly inherited gain-of-function mutation, I693T, causing paramyotonia congenita (Matthews *et al.*, 2008). Importantly, the report in this issue by Zaharieva and colleagues adds recessive mutations of $Na_v1.4$ as another possible cause of

congenital myopathy, especially when neonatal hypotonia with breathing and swallowing difficulties is present. This study also establishes the existence of null alleles for $Na_v1.4$ in humans and illustrates the differential consequences of inheriting a hypomorphic loss-of-function allele versus a complete null.

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Hypoxia, a turning point in migraine pathogenesis?

This scientific commentary refers to 'Migraine induced by hypoxia: an MRI spectroscopy and angiography study', by Arngrim *et al.* (doi:10.1093/brain/awv359).

Experimental models of human diseases are vital for pathophysiological and therapeutic research. In migraine, animal models have contributed greatly to better understanding of causal mechanisms and to the development of novel therapies, but they do not allow comprehensive analysis of the entire clinical phenotype. Human models are more suitable for this purpose, particularly because there are no insurmountable ethical barriers to inducing migraine attacks as they produce no sequelae and are treatable. The nitroglycerin test is the best studied human model for inducing attacks in migraine patients, although various other compounds have a similar effect (Olesen, 2008). The sensitivity of the nitroglycerin test is up to 80% in migraine without aura. However, nitroglycerin only rarely induces a migrainous headache in patients with migraine with aura and almost never an aura (Sances *et al.*, 2004). A human model for the induction of migraine with aura attacks is thus still missing. In this issue of *Brain*, Arngrim and co-workers demonstrate that exposure to hypoxia may be one such model (Arngrim *et al.*, 2016).

There are several reasons why normobaric hypoxia might be suitable. First, there is evidence that migraine (with aura) attacks may be triggered when blood oxygen saturation decreases. High-altitude headache occurs frequently with ascent above 2500 m, has at least some migrainous features and is more prevalent in mountaineers with a history of migraine (ICHD-3b, code 10.1.1 2013). Moreover, sleep apnoea-induced migraine attacks improve after bariatric surgery (Kallweit *et al.*, 2011). Second, it has been known for more than two decades from studies *in vitro* and *in vivo* that hypoxia can induce spreading depression (Somjen *et al.*, 1992), the most likely cortico-subcortical generator of the migrainous aura, thought to be favoured by increased brain glutamate levels. Third, mitochondrial energy metabolism may be deficient in migraine with and without aura between attacks, a frequently neglected facet of migraine pathophysiology that could sensitize migraineurs to hypoxia (Paemeleire and Schoenen, 2013). That metabolic failure might play a crucial role in migraine was hypothesized by Amery in 1982, before the first experimental data supporting the hypothesis were obtained by studies using ^{31}P magnetic resonance spectroscopy (MRS). The most recent of these used improved methodology and found that ATP is decreased by 16% and phosporylation potential by 39% in migraine patients between attacks (Reyngoudt *et al.*, 2011). Interestingly, experimental mitochondrial poisoning also induces cortical spreading depression and facilitates hypoxia-induced spreading depression in rat hippocampal slices. Fourth, the