Review article

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Myoclonus epilepsy and ataxia due to potassium channel mutation (MEAK) is caused by heterozygous *KCNC1* mutations

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ABSTRACT – Progressive myoclonus epilepsy (PME) is a distinct group of seizure disorders characterized by gradual neurological decline with ataxia, myoclonus and recurring seizures. There are several forms of PME, among which the most recently described is MEAK - myoclonus epilepsy and ataxia due to potassium channel mutation. This particular subtype is caused by a recurrent *de novo* heterozygous mutation (c.959G>A, p.Arg320His) in the *KCNC1* gene, which maps to chromosome 11 and encodes for the Kv3.1 protein (a subunit of the Kv3 subfamily of voltage-gated potassium channels). Loss of Kv3 function disrupts the firing properties of fast-spiking neurons, affects neurotransmitter release and induces cell death. Specifically regarding Kv3.1 malfunctioning, the most affected neurons include inhibitory GABAergic interneurons and cerebellar neurons. Impairment of the former cells is believed to contribute to myoclonus and seizures, whereas dysfunction of the latter to ataxia and tremor. Phenotypically, MEAK patients generally have a normal early development. At the age of 6 to 14 years, they present with myoclonus, which tends to progressively worsen with time. Tonic-clonic seizures may or may not be present, and some patients develop mild cognitive impairment following seizure onset. Typical electroencephalographic features comprise generalized epileptiform discharges and, in some cases, photosensitivity. Brain imaging is either normal or shows cerebellar atrophy. The identification of MEAK has both expanded the phenotypic and genotypic spectra of PME and established an emerging role for *de novo* mutations in PME.

Key words: MEAK, *KCNC1*, progressive myoclonus epilepsies, myoclonus, seizures, potassium channel mutations, ataxia

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The term 'progressive myoclonus epilepsy' (PME) (Minassian *et al*. 2016) provides us with a broad, but reliable, characterization for this distinct subgroup of seizure disorders. 'Progressive' depicts the gradual neurological decline seen in patients affected with a PME.

'Myoclonus' and 'epilepsy' summarize the other two cardinal clinical features observed in these individuals (Berkovic *et al*., 1986). Aetiologically, most PMEs are the result of genetic abnormalities. The vast majority are inherited in an autosomal recessive fashion, although some cases exhibit autosomal dominant or mitochondrial inheritance.

Despite the impressive range of specific forms of PME, their phenotypes are fairly similar, which makes accurate clinical diagnosis very challenging, especially in the early stages. However, significant heterogeneity exists in terms of the genetic causes of the subtypes of progressive myoclonus epilepsies.

As is the case with countless other diseases in the realm of neurology, a proportion of PME cases still have no identified genetic basis. In this context, a cohort of 84 unconfirmed, unrelated PME patients was extensively investigated in a recent multicentre study. With a view to trying to elucidate their molecular diagnoses, all cases underwent whole-exome sequencing, and Sanger sequencing was performed for a secondary cohort of 28. The most remarkable finding was the identification of a recurrent heterozygous *de novo* mutation in the *KCNC1* gene, which encodes for a subunit of a specific potassium channel. The new PME subtype was named 'myoclonus epilepsy and ataxia due to potassium channel mutation' (MEAK) (Muona *et al*., 2015). Notably, prior to this study, *KCNC1* had never been associated with any human disease. In addition, this group of researchers importantly demonstrated that *de novo* mutations play a significant role in the genesis of progressive myoclonus epilepsies.

This article aims to review the most recent PME subtype described: MEAK. We begin by introducing the microworld of potassium channels, since an understanding of this is of fundamental importance. We then focus on how *KCNC1* was identified to be associated with MEAK and discuss how the effects of such a mutation are translated into clinical signs and symptoms. Finally, the phenotypes of all the reported individuals with MEAK are discussed.

Potassium ion channels

Potassium ion channels are ubiquitous membrane proteins responsible for a range of cellular functions, including maintenance of membrane potential, regulation of cell volume, and electrical excitability modulation. The latter is particularly important for the membrane physiology of excitable cells, such as neurons. According to their functional properties, potassium channels can be categorized into several groups, such as **voltage-gated potassium channels**, calcium-activated potassium channels, and sodium-activated potassium channels, among others. Furthermore, each of these groups can be subdivided into families and subfamilies, based on molecular similarity (Sansom *et al*., 2002).

Voltage-gated potassium channels (Kv channels) play an essential role in the generation and propagation of electrical impulses in the nervous system. By enabling the selective flow of potassium ions through neuronal membranes (upon changes in transmembrane potentials), Kv channels help set the resting potential and degree of excitability of the membrane (repolarization), influence action potential waveforms and firing patterns, and modulate synaptic activity (Ried *et al*., 1993). Voltage-gated potassium channels are classified into four subfamilies: Kv1, Kv2, Kv3, and Kv4.

Kv3 channels, in particular, are known for their high activation threshold and fast activation and deactivation properties (Rudy & McBain, 2001). Kv3 channels are crucial components of the circuitry of neurons that are able to fire action potentials at high frequencies or follow high frequency inputs (Wang *et al*., 2007). The Kv3 subfamily is composed of four subunits, Kv3.1, Kv3.2, Kv3.3, and Kv3.4, which are encoded by four genes, *KCNC1*to *KCNC4*, respectively.

These four subunits assemble as either homomers or heteromers to form voltage-gated tetrameric potassiumion channels. Each of the four subunits consists of 6 transmembrane segments (S1-S6) with a re-entrant P-loop region. The transmembrane segments S1-S4 are referred to as the voltage-sensing domain, of which the primary voltage-sensing unit is S4. The segments S5-P-S6 represent the ion-conducting pore domain. Upon membrane de- or hyper-polarization, which is sensed by positively charged arginine residues at the S4 segment, the S4 segment undergoes the largest reorientation, leading to channel opening and generation of transient gating currents (Aggarwal & MacKinnon, 1996; Chanda & Bezanilla, 2008) (*figure 1*).

Within the *KCNC* gene family, *KCNC3* has been previously reported as a human disease associated gene. *KCNC3* mutations have been recognized as a cause of spinocerebellar ataxia type 13 (*SCA13*) (Herman-Bert *et al*., 2000; Middlebrooks *et al*., 2013). Only very recently, *KCNC1* mutations have been identified as a cause of human disease: myoclonus epilepsy and ataxia due to potassium channel mutation (MEAK).

Discovering *KCNC1* mutations in myoclonus epilepsy and ataxia (MEAK)

Eighty-four clinically confirmed cases of PME without clear genetic aetiology from multiple centres (including Europe, North America, Asia, and Australia), were investigated using whole-exome sequencing. A recurrent heterozygous missense mutation, c.959G>A

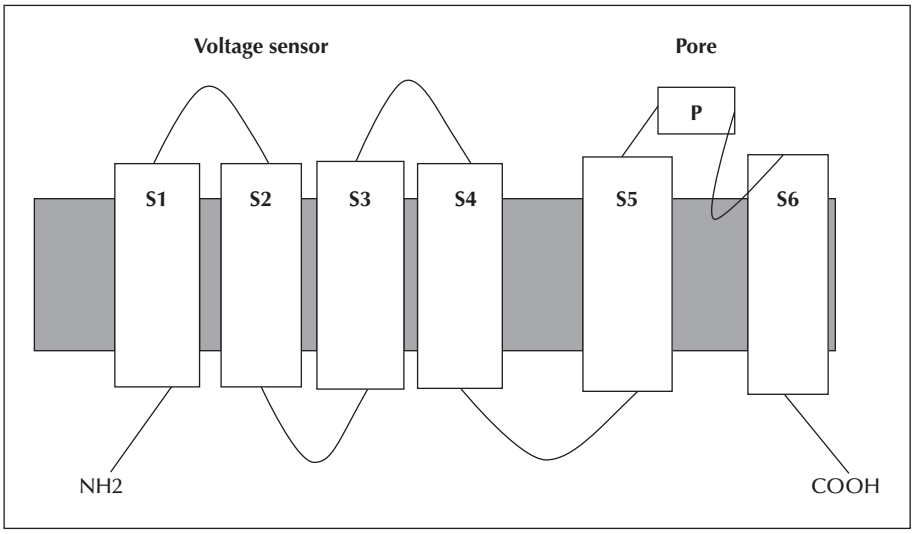

Figure 1. Transmembrane topology of a Kv channel subunit.

(p.Arg320His), in *KCNC1* was identified in 11 unrelated patients (13.1 per cent). Sanger sequencing confirmed the mutation in two new cases, as well as in an affected sister and two affected children of one of the original 11 cases. In total, 16 cases, 13 of them unrelated, had the same *KCNC1* mutation. The parents of all patients with the *KCNC1* mutation were unaffected. Segregation analysis, where DNA was available for both parents, revealed that in each case the mutation occurred *de novo*. The family with four MEAK cases was further evaluated to rule out parental mosaicism (Muona *et al*., 2015). Based on a recently published mutation model (Samocha *et al*., 2014), the rate of this specific mutation was estimated at 1.75×10^{-7} mutations per person, representing an occurrence in one out of every 5,700,000 conceptions.

Pathogenicity of *KCNC1* mutations

KCNC1 maps to chromosome 11, at 11p15.1, and encodes the Kv3.1 potassium channel (Ried *et al*., 1993). The c.959G>A mutation causes a substitution of histidine for arginine at codon 320 of the Kv3.1 protein (p.Arg320His). The targeted arginine is a highly evolutionary conserved residue in segment S4, which constitutes the main voltage sensor of Kv3 channels. Functional analysis showed that, upon membrane depolarization, mutant channels produced hardly detectable currents (Muona *et al*., 2015). Interestingly, experiments also indicated a dominant-negative effect of the mutant channel. When the mutant subunit and the wild-type protein were co-expressed at a ratio of 1:1, an 80 per cent decrease in the expected current was observed (Muona *et al*., 2015).

Previous studies have shown that loss of Kv3 function (such as that caused by p.Arg320His in *KCNC1*) disrupts the firing properties of fast-spiking neurons (Erisir *et al*., 1999; Issa *et al*., 2011; Rudy & McBain, 2001), affects neurotransmitter release (Sabatini & Regehr, 1997), and induces cell death (Irie *et al*., 2014). Specifically, in terms of Kv3.1 malfunctioning, the most affected neurons include inhibitory GABAergic interneurons (Gan & Kaczmarek, 1998; Rudy & McBain, 2001) and cerebellar neurons. Consequently, within the context of a p.Arg320His mutation, the physiology of both these types of neuronal cells would be compromised and could, eventually, degenerate. The implications of these outcomes are useful in attempting to explain the clinical scenario of subjects with MEAK. The first implication would be disinhibition following impaired firing of fast-spiking GABAergic interneurons, which is believed to contribute to myoclonus and tonic-clonic seizures. The second implication would be the dysfunction and degeneration of cerebellar neurons, which is hypothesized to contribute to motor impairment and ataxia.

MEAK phenotypes

The phenotypes of the 16 patients with MEAK were fairly similar. Early development was, as a rule, normal. The first symptom tended to be myoclonus, with an onset ranging from the age of 6 to 14 years. Myoclonus progressively worsened. Gait disturbances due to myoclonus led to the use of a walking aid or wheelchair by adolescence or early adulthood in most patients. Tonic-clonic seizures were present, albeit infrequent. Learning difficulties before seizure onset were not common. Mild cognitive decline subsequent to seizure onset, nonetheless, was shared by roughly half of the individuals. On electroencephalogram, these patients had generalized epileptiform

discharges, with photosensitivity in some cases. Magnetic resonance imaging was either normal or showed cerebellar atrophy.

The early clinical presentation and evolution of MEAK resembles that of Unverricht-Lundborg disease (ULD). The age at onset, moderate-to-severe myoclonus, infrequent tonic-clonic seizures, and mild, if any, cognitive decline, are characteristic of both MEAK and ULD. As MEAK evolves, however, it might be clearly distinguished from ULD, as patients with MEAK usually suffer a more severe course. In terms of molecular diagnosis, however, the two entities are easily differentiated, irrespective of the time of diagnosis. While MEAK is caused by *KCNC1* mutations, ULD is most frequently a result of mutations in the promoter region of *CSTB*, which leads to a massive reduction in levels of cystatin B, a lysosomal protease inhibitor (Rinne *et al*., 2002; Girard *et al*., 2013).

Conclusion

The identification of MEAK and its genetic basis have widened the phenotypic and genotypic spectra of PME. Given that pathogenic *KCNC1* mutation is estimated to affect one in every 5,700,000 conceptions, many undetermined PME cases worldwide may now be solved. Diagnosing these individuals is likely to have a direct impact on counselling and education for patients and caregivers.

In addition, an emerging role for *de novo* mutations in progressive myoclonus epilepsies has been established. Finally, an understanding of the pathophysiology of MEAK provides singular insights into potential therapeutic interventions. □

Disclosures.
None of the authors have any conflict of interest to disclose.

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