A NOVEL KCNA1 MUTATION CAUSING EPISODIC ATAXIA TYPE I

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ABSTRACT: We describe the clinical phenotype of a novel de novo KCNA1 mutation, and functional characterization of the effects of the mutation on Kv1.1 channel function. HEK293 cells were transfected transiently with either wild-type or mutant channels. Representative currents were evoked after application of a series of square voltage steps from $-80$ mV to $+50$ mV in 200-ms intervals from $V_0 = -80$ mV. Extracellular K$^+$ was added to evoke tail currents. Equal amounts of wild-type and Kv1.1,1262M mutant DNA were transfected transiently in HEK293 cells to evaluate the influence of the mutation. We found that Kv1.1,1262M leads to a defective voltage-gated potassium channel. Coexpression studies revealed a dominant-negative effect. We describe the phenotype of a novel KCNA1 mutation causing episodic ataxia. Patch-clamp studies confirm the pathogenicity of the mutation in vitro and suggest that it is dominant with respect to wild-type.


EPISODIC ATAXIA TYPE 1 (EA1) is a rare autosomal dominant ion channelopathy that usually presents in the first to second decades of life.¹ It is caused by mutations in the KCNA1 gene located on chromosome 12p13, which encodes the α-subunit of the fast voltage-gated potassium channel Kv1.1.² In myelinated peripheral nerve, Kv1.1 mediates neuronal repolarization by limiting axonal hyperexcitability after the action potential.¹,³ EA1 is characterized by attacks of ataxia and continuous myokymia, a form of spontaneous muscle activity caused by peripheral nerve hyperexcitability.¹

REFERENCES
We present the clinical and physiological characteristics of a novel KCNA1 mutation.

CASE REPORT
This patient had experienced classic EA1 symptoms since early childhood. At 30 years of age, he developed a slowly progressive, generalized tremor. At age 35, he began having generalized stiffness due to increased muscle tone. The patient’s neurologic examination at age 40 is shown in the video in the Supplementary Material (available online). Two years later he developed lower limb spasticity with hyperreflexia and extensor plantar responses. Family history was negative for EA1 and other ion channelopathies, such as seizures or migraine. Needle electromyography showed generalized myokymic discharges. Nerve conduction studies, laboratory investigations, electroencephalography, and neuraxis magnetic resonance imaging data were normal. Sodium valproate, 3,4-diaminopyridine, and phenytoin were ineffective. Acetazolamide and carbamazepine worsened tremor. Gabapentin and clonazepam ameliorated muscle stiffness but not tremor.

Mutation Analysis. The KCNA1 gene was screened for mutations by DNA sequence analysis of the complete coding region, including exon boundaries. A heterozygous nucleotide change of a cytosine to guanine at position 786 of exon 1 of the KCNA gene (c.786C>G) was found, resulting in substitution of an isoleucine amino acid at position 262 of the KCNA1 protein by a methionine (I262M) in the third transmembrane segment of the alpha unit. Neither of the proband’s parents carried this mutation.

METHODS
The patient authorized release of the aforementioned video. Electrophysiological methods and solutions are described extensively in the Supplementary Material. In brief, human embryonic kidney 293 (HEK293) cells transiently expressing either wild-type (WT) or mutant channels were used for functional studies under a whole-cell configuration patch-clamp technique. Representative currents were evoked after application of a series of square voltage steps from $-80 \text{ mV}$ to $+50 \text{ mV}$ in 200-ms intervals from $V_h = -80 \text{ mV}$. Tail
currents were evoked by adding 50 mM extracellular K+.

Equal amounts of wild-type and Kv1.1262M mutant DNA were transfected transiently in HEK293 cells to evaluate the dominance of the identified mutation. Data are presented as mean ± standard error of the mean (SEM). Statistical analysis was performed with 1-way analysis of variance (ANOVA) followed by the Bonferroni post hoc correction. P < 0.05 was considered significant.

RESULTS

Functional Characterization of the Kv1.1262M Mutant. Cells expressing WT Kv1.1 evoked fast delayed rectifying currents at depolarizing potentials that were significantly (P < 0.05) reduced on Kv1.1262M-expressing cells (Fig. 1A and B). Density currents were 186 ± 42 pA/pF and 14 ± 3 pA/pF for WT and the mutant, respectively (Fig. 1C). Current density obtained for the mutant was not significantly different from that of the mock conditions (17 ± 3 pA/pF). This result strongly suggests that the mutation found in this patient results in a non-functional homomeric channel, as surface expression experiments showed no significant changes in trafficking as a consequence of the mutation (Fig. 1D).

Kv1.1262M Mutation Exerts a Dominant-Negative Effect on WT Kv1.1 Function. To study function of heterotetramers consisting of WT and Kv1.1262M subunits, we performed coexpression experiments where equal amounts of DNA were transfected transiently in HEK293 cells. Outward currents obtained when both subunits were present resembled the situation from either mock or homomeric Kv1.1262M-mediated currents (Fig. S1A and B in Supplementary Material). This represents a significant reduction in the density current when compared with the WT/WT or the WT/mock condition without affecting the surface expression of the potassium channel (Fig. S1C and D in Supplementary Material), thereby supporting the hypothesis that the mutation leads to a dominant-negative effect with respect to WT.

DISCUSSION

We describe a novel de novo I262M mutation in the KCNA1 gene, which is associated with an episodic ataxia phenotype with generalized tremor, muscle stiffness, and lower limb spasticity.

Electrophysiological characterization of the Kv1.1262M mutant confirmed a dominant-negative effect, which leads to a non-functional ion channel. Accordingly, the episodic attacks of ataxia and myokymia found in this patient would be the result of increased neuronal excitability as a consequence of defective membrane repolarization after an action potential. A compensatory effect mediated by other isoforms (i.e., Kv1.2) may account for the transient episodes of ataxia.

Kv channels are tetrameric structures, in which each subunit consists of 6 transmembrane-spanning α-helix and cytoplasmic N- and C-terminal domains. These channels respond to membrane depolarization due to the presence of a voltage sensor comprising S1–S4 segments. Although S4 contains the positively charged amino acids that serve as the gating charges, the adjacent S3 segment also plays an important role in gating. Our study contributes a new de novo missense mutation found in S3, which supports this conclusion. Coexpression studies showed that our missense Kv1.1262M mutant resulted in an 85% current amplitude reduction (complete loss of function when expressed alone), whereas a previous study showed a 60% reduction in a Kv1.1262T mutant (15% residual current when expressed alone). Despite phenotypic differences, neuromyotonia was a common symptom in both patients. Those results suggest that mutations in relevant S3 amino acid positions produce severe gating impairment by affecting conformational changes, most likely by uncoupling of both S4 and S3 segments.

The patient we describe responded poorly to drugs that are often used in EA1. Disease severity and residual channel function appear to correlate with treatment response (Table S1 in Supplementary Material). Perhaps this indicates that residual Kv1.1 function is required to elicit a therapeutic response.

One other de novo KCNA1 mutation has been described previously. Although rare, the possibility of a de novo mutation must be considered during the clinical evaluation and genetic counseling of neuromyotonia patients.

In conclusion, we have shown that the novel KCNA1 mutation I262M leads to a non-functional potassium channel, which accounts for an EA1 phenotype with generalized tremor, muscle stiffness, and lower limb spasticity.

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REFERENCES