A Novel *KCNA1* Mutation in a Family with Episodic Ataxia and Malignant Hyperthermia

Tiago A. Mestre[1,2], Andreea Manole[3], Heather MacDonald[4], Sheila Riazi[5], Natalia Kraeva[5], Michael G Hanna[3,] Anthony E Lang[1], Roope Männikkö[3]**, Grace Yoon[4,6]*

1 Morton and Gloria Shulman Movement Disorders Clinic and the Edmond J. Safra Program in Parkinson's Disease, Toronto Western Hospital, University Health Network, Division of Neurology, Department of Medicine, University of Toronto, Toronto, Canada

2 Parkinson's Disease and Movement Disorders Center, Division of Neurology, Department of Medicine, The Ottawa Hospital Research Institute, University of Ottawa, Canada (Current affiliation)

3 MRC Centre for Neuromuscular disease, UCL Institute of Neurology, Queen Square, London, United Kingdom

4 Division of Clinical and Metabolic Genetics, The Hospital for Sick Children, University of Toronto, Toronto, Canada

5 Malignant Hyperthermia Investigation Unit, Toronto General Hospital, Department of Anesthesia, University of Toronto, Toronto, Ontario, Canada

6 Division of Neurology, The Hospital for Sick Children, University of Toronto, Toronto, Canada

Corresponding authors:

Grace Yoon

Division of Clinical and Metabolic Genetics

555 University Avenue

Toronto, Ontario, Canada

M5G 1X8

Email: grace.yoon@utoronto.ca

Roope Männikkö

MRC Centre for Neuromuscular Disease, Department of Molecular Neuroscience

UCL Institute of Neurology

Queen Square House

London, WC1N 3BT

United Kingdom

Email: r.mannikko@ucl.ac.uk

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ABSTRACT

Episodic ataxia type 1 (EA1) is an autosomal dominant channelopathy caused by mutations in *KCNA1,* which encodes the voltage-gated potassium channel, Kv1.1. Eleven members of an EA family were evaluated with molecular and functional studies. A novel c.746T>G (p.Phe249Cys) missense mutation of *KCNA1* segregated in the family members with episodic ataxia, myokymia and malignant hyperthermia susceptibility. No mutations were found in the known malignant hyperthermia genes *RYR1* or *CACNA1S*. The Phe249Cys-Kv1.1 channels did not show any currents upon functional expression, confirming a pathogenic role of the mutation. Malignant hyperthermia may be a presentation of *KCNA1* mutations, which has significant implications for the clinical care of these patients and illustrates the phenotypic heterogeneity of *KCNA1* mutations.

INTRODUCTION

Episodic ataxia type 1 (EA1) is an autosomal dominant channelopathy characterized by brief episodes of cerebellar ataxia and dysarthria. [1] Interictal myokymia is typically reported in EA1, but persistent cerebellar features have also been described. [2] EA1 is caused by loss-of-function mutations in the *KCNA1* gene encoding the voltage-gated potassium (K^+) channel, Kv1.1 that lead to neuronal hyperexcitability. [3] The onset is typically in early childhood and episodes of ataxia are usually precipitated by physical and emotional stress, startle, or sudden movements. [1] Clinical heterogeneity associated with *KCNA1* mutations has been reported including isolated muscle spasms with rigidity [4], hearing impairment [5], cerebellar atrophy, cognitive delay, myokymia and epilepsy [6, 7] migraine [8] and distal lower limb weakness and stiffness.[9] Loss of Kv1.1 channel function leads to neuronal hyperexcitability and prolonged duration of action potentials. [5] Malignant hyperthermia (MH) is a pharmacogenetic disorder of the skeletal muscle, secondary to abnormal cellular calcium homeostasis, which has been associated with mutations in the skeletal muscle ryanodine receptor (*RYR1*) and α1 subunit of the DHPR (*CACNA1S*). [10] We report a large family with a history of EA1 and MH that segregated with a novel missense mutation of *KCNA1,* further expanding the clinical phenotype associated with *KCNA1* mutations.

MATERIALS AND METHODS

Subjects. The proband (Subject III-15) and ten family members were assessed using a standardized protocol after providing written informed consent. This study was approved by the Research Ethics Board of the Hospital for Sick Children.

Mutation screening. All subjects in generation II were screened for mutations in *KCNA1* (MIM# 176260). Additionally, screening of the entire coding regions of *RYR1* (MIM# 180901) and *CACNA1S* (MIM# 114208) was performed in Subject II-6, and exome

sequencing was performed for the proband (Subject III-15) and her father (Subject II-6). Direct Sanger and exome sequencing were performed at The Centre for Applied Genomics (TCAG), Toronto, Canada.

Functional Studies (available as Supplemental Data)

RESULTS

Subjects. *Subject III-15 (Figure 1, Table 1).* This 18-year old female had a history of recurrent episodes of gait unsteadiness since age 10, with progression of the episodes frequency since age 13. The episodes lasted between 10-60 minutes and were triggered by sudden unexpected stimuli, but also occurred at night. The episodes were preceded by a sensation of warmth spreading over the whole body, followed by a feeling of inebriation and incoordination of the limbs together with slurred speech, blurry vision and vertigo. Stress was associated with more severe episodes. A post-episode headache was usually present. The exam between episodes was normal apart from upper limbs overshooting during mirror testing and a flickering motion of the fingers. Acetazolamide, carbamazepine, lamotrigine, and topiramate were of no benefit. Routine cardiac (EKG, echocardiogram and 24-h Holter monitor), metabolic (organic acids, amino acids) and neurological (EMG/NCS, EEG, MRI) investigations were normal. Genetic testing of *CACNA1A, SCN1A, ATP1A2, CACNB4* and *SLC1A3* was negative. Exome sequencing did not reveal variants which were bioinformatically predicted to be damaging in any other genes.

The father of Subject III-15 had a history compatible with episodic ataxia and tested positive for MH susceptibility using the caffeine/halothane contracture test (CHCT) on skeletal muscle biopsy. His brother (Subject II-5) passed away at age 1 year after a surgery due to cardiac arrest in the setting of MH post anesthesia, without administration of dantrolene. Genetic testing of the two genes known to cause the majority of MH susceptibility, *RYR1* and

CACNA1S, was negative, as was exome sequencing. Additional clinical histories of other family members are available as supplemental data.

Mutation Identification for EA. All family members with symptoms consistent with episodic ataxia carried the c.746T>G (p.Phe249Cys) mutation (Figure 1). The mutation also segregated with family members who had a confirmed diagnosis of MH susceptibility (Table 1).

Functional analysis. Upon expression of p.Phe249Cys channels in *Xenopus* oocytes, K^+ currents could not be detected (Figure 2). Wild-type (WT) channels produced robust currents. In simulated heterozygous condition the K^+ currents were readily detectable and the voltage dependent-activation was similar to the WT channels ($p=0.082$, Figure 2C). The tail current amplitude was ~1/2 of those in oocytes injected with wild-type mRNA only (same total amount of mRNA) (Figure 2). To test if the p.Phe249Cys subunits could form heterotetramers with WT subunits we made the p.Phe249Cys channel insensitive to TEA block by introducing a Tyr379Val mutation to the same polypeptide as the p.Phe249Cys mutation (Phe249-Tyr379Val). [11] When co-expressed together with WT subunits in 1:1 ratio, the sensitivity of WT/Phe249Cys-Tyr379Val channels was reduced ($p<0.001$) (Figure 2D). However, this reduction in TEA-sensitivity was much smaller than when the wild-type channels were co-expressed with wild-type subunits made insensitive to TEA block (Figure 2D). To test if the co-expression of p.Phe249Cys may negatively influence the function of the WT subunits we injected an increasing amount of Phe249Cys mRNA at a constant amount of WT mRNA. As the concentration of p.Phe249Cys mRNA increased, the current amplitude decreased (Figure 2E).

DISCUSSION

We report a novel c.746T>G (p.Phe249Cys) mutation of *KCNA1* co-segregating in a two-generation family clinically diagnosed with EA or myokymia and MH susceptibility. The Phe249 amino acid residue is located in the intracellular loop between transmembrane segments TM1 and TM2 within the voltage-sensing domain of the Kv1.1 channel. It is highly conserved across species indicating that the phenyalanine at position 249 is essential for normal channel function. The functional studies further support the pathogenicity of the p.Phe249Cys mutation by demonstrating a complete loss-of-function in the homozygous state. In the heterozygous state, the K^+ current amplitude reduced to half of wild type channels and voltage dependence of channel activation was unaltered. These data are consistent with those of the previously characterized mutation affecting the same residue, p.Phe249Ile. [11] In addition, the electrophysiology data suggests that the mutant subunit can co-assemble with the wild-type subunits and may reduce the functional expression of the heterotetramer in the cell surface. Of interest, in all family members in whom MH susceptibility was documented, the p.Phe249Cys mutation segregated with positive CHCT results. In Subject II-6, genetic testing of *RYR1* and *CACNA1S* followed by exome sequencing was negative. These findings raise an important question regarding a potential contribution of the p.Phe249Cys *KCNA1* mutation to MH in this family. The fatal MH event in Subject II-5 may have been caused by functionally impaired Kv1.1 channels precipitated by exposure to anesthesia. Volatile anesthetics are known to have an inhibitory effect on Kv1.1 channels [12], and *KCNA1* knock-out mice have muscle hyperexcitability. [13] The effect of volatile anesthetics on a mutated channel with reduced current could lead to increase in excitability of the peripheral nervous system, which expresses Kv1.1 channels, and secondarily maintain cardiac and skeletal muscle contraction with dysregulation of the Ca^{2+} homeostasis. Loss-of-function mutation in Kv1.1 channels may potentially induce a thermoregulatory defect in hypothalamic neurons that mediate rapid core body temperature

changes [8], as seen in MH. We postulate that the p.Phe249Cys *KCNA1* mutation could have precipitated the MH event in this family.

In conclusion, we report a novel *KCNA1* mutation associated with an EA1 phenotype and a possible association with MH. The current report broadens the phenotypes associated with *KCNA1* mutations to include possible susceptibility to MH.

AUTHOR CONTRIBUTIONS:

TAM – took care of the patients, was responsible for the study concept and design, acquisition of data, analysis and interpretation, and wrote the first draft of the manuscript.

AM, MGH, and RM - were responsible for the electrophysiological studies and contributed Figure2.

HM – contributed clinical data and Figure 1.

SR, NK – were responsible for the clinical and genetic evaluations of malignant hyperthermia for the patients.

AEL - contributed clinical data and data analysis.

GY – took care of the patients, was responsible for the study concept and design, acquisition of data, analysis and interpretation, and overall supervision of the study.

All authors critically revised the manuscript for important intellectual content.

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ETHICAL APPROVAL: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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FIGURE LEGENDS

Figure 1. Family Pedigree. Horizontal bars denote individuals with EA. Vertical bars denote individuals with MH. Phe249Cys *KCNA1* mutation status is indicated as "+" or "-".

Figure 2. Electrophysiological characterization of Phe249Cys Mutant channels. K^+ currents after expression of the Wild-type (WT) channels (A) or of the Phe249Cys channels (B) on *Xenopus laevis* oocytes. Capacitive transients at beginning of the voltage steps are not shown. (C) Current-Voltage relationship of WT (n=27), Phe249Cys (F249C, n=12) and heterozygous Phe249Cys channels (F249Chet, n=23). Solid lines show fit of Boltzman equation to the mean data. (D) Mean TEA dose-response data for *Xenopus laevis* oocytes injected with WT (n=10), TEA insensitive WT (WT-TEA, n=4), a 1:1 mix of WT and WT-TEA (n=8) or a 1:1 mix of WT and TEA insensitive Phe249Cys mRNA (F249C-TEA, n=6). Solid lines show fit of Equation 2 to the mean data.
(E) Mean current-voltage data for *Xenopus leavis* oocytes injected with 100 ng (WT*2, n=27) or 50 ng WT mRNA (WT, n=22), 50 ng WT and 50 ng Phe249Cys mRNA (WT+F249C, n=23), or 50 ng WT and 200 ng Phe249Cys mRNA (WT+F249C*4, n=17). Solid lines show fit of Boltzman equation to the mean data.