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

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Supplemental Data

Case Histories

Subject II-6. This 50-year-old man, father of Subject III-15, had a history of recurrent episodes of gait unsteadiness since childhood with episodes no more than 5 minutes in duration and triggered by tripping when curling or playing hockey. The intensity of the episodes was mild and never disrupted his motor function. The neurological examination at age 50 was only remarkable for a mild intention hand tremor bilaterally. He had a significant family history of malignant hyperthermia. 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. The remaining 5 siblings were tested for MH susceptibility using the caffeine/halothane contracture test (CHCT) on skeletal muscle biopsy, and all except one (Subject II-3) had a positive diagnosis. Genetic testing of the two genes known to cause the majority of MH susceptibility, *RYR1* and *CACNA1S*, was negative.

Subject II-1 This 49-year old woman had a longstanding history of muscle cramps and hand twitching since childhood. There are no particular triggers for these symptoms and they could occur at rest. She had a history of recurrent episodes of dizziness and unsteadiness starting at age 31. The episodes lasted approximately 5 minutes and were followed by a headache. In addition, she had a history of three episodes of generalized tonic clonic seizures seizure at age of 33, confirmed by electroencephalogram. She was on anti-epileptic medication since then (phenytoin

and carbamazepine). There were no other medical problems. The neurological examination at age 49 was remarkable for a mild intention hand tremor bilaterally.

Subject III-3 This 26-year old woman had a history of recurrent episodes of unsteadiness triggered by exercise. The episodes started with a feeling of numbness in the tongue followed by a "jelly" sensation in the legs, a leg tremor, leading to imbalance to the point she usually needs support. These episodes lasted approximately 30 seconds in duration and resolved spontaneously. The frequency of episodes was variable ranging from every two weeks to several months free of episodes. In addition, she had a long-standing history of muscle cramps beginning early in childhood and resolving in adolescence, as well as a rippling sensation of her muscles starting during childhood. The neurological examination was only remarkable for a slight hand action tremor bilaterally.

Subject III-4 This 22-year old woman had recurrent episodes of dizziness, unsteadiness, and imbalance with the need to support herself for the previous 10 years. The episodes lasted approximately 5 minutes and occurred on a monthly basis. In addition, she had a 1-year history of hand cramps lasting several minutes in duration with frequency of occurrence ranging from every two weeks to every few months. The episodes did not appear to have specific triggers. She described herself as always being clumsy as a child, and for that reason always avoided sport activities. The neurological examination was only remarkable for a slight hand postural tremor bilaterally.

Subject III-8. This 22-year old young man had episodic recurrent twitching in the fingers of both hands with onset at adolescence that would last for less than 1 minute without a known trigger. In addition, his history was remarkable for sporadic nocturnal muscle cramps. There was

no report of episodes of unsteadiness, seizures or headaches. Neurological examination was normal.

Exome Sequencing

Whole exome capture and sequencing were performed for 2 members of the family (the proband and her father). 2 X 100bp paired end sequencing was done using the Illumina Hi-Seq 2000 platform after target enrichment of 6.5µg of genomic DNA with the Agilent SureSelectHuman All Exon 50Mb Capture kit. Sequence reads were aligned to the reference human genome (hg19/GRCh 37) with Burrows-Wheeler aligner, and paired-end duplicate reads were removed (MarkDuplicates, Picard tools v.1.35; http://picard.sourceforge.net/). Single nucleotide variants (SNVs) and insertions/deletions (indels) were detected using GATK 1.1.28 annotated using SNPEff (http://snpeff.sourceforge.net/). Variants were also annotated for frequency in public databases [1,000 genomes; dbSNP135, http://www.ncbi.nlm.nih.gov/projects/SNP/; NHLB1 Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/].

Functional Studies. *Molecular biology.* Mutations were introduced into the human *KCNA1* cDNA in pMTLF plasmid using Quick-change site-directed mutagenesis kit (Qiagen). Successful mutagenesis was confirmed by sequencing over the mutated site. The mRNA was synthesized *in vitro* using mMessage machine kit (Agilent). Two separate mRNA preparations were tested for p.Phe249Cys mutation. *Oocyte preparation.* Oocytes were isolated from adult female *Xenopus laevis* following procedures that have been approved by UCL's Biological Services Management Group and the UK Home Office. *Xenopus leavis* oocytes were injected with 50 nl mRNA (5-25 ng) and incubated at 15°C in Modified Barth's Solution containing (in mM): 88 NaCl,1 KCl, 1.68 $MgSO_4$, 10 HEPES, 0.47 $Ca(NO_3)_2$, 2.4 $NaHCO_3$, 0.41 $CaCl_2$ (pH 7.4) with 50 U/ml penicillin G and 50 mg/ml streptomycin.

Electrophysiology. The K^+ currents were recorded with a two-electrode voltage clamp using GeneClamp 500 amplifier (Axon Instruments, Foster City, CA, USA) at room temperature (22–24^{O}C) with continuous superfusion of oocytes with ND96 (in mM): 96 NaCl, 2 KCl, 1 $MgCl_2$,1.8 $CaCl_2$, 5 HEPES (pH 7.4). A TEA^+-stock solution (in mM: 96 TEACl, 2 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 HEPES) was diluted in ND96 to yield TEA test concentrations between 0.03 and 30 mM. Glass electrodes (GT150, Harvard apparatus) filled with 3 M KCl had resistances between 0.1 and 1 MΩ. Data were acquired using Clampex 10.1 (molecular devices) and analysed using Clampfit 10.1 (Molecular devices), Excel (Microsoft) and Origin (Microcal) software. Voltage dependence of activation was determined by clamping the oocyte to test voltages ranging from -120 mV to 90 mV in 10 mV increments from holding voltage of -90 mV, followed by a tail voltage step to -40 mV. The current ~4 ms after the onset of the tail step, following the settling of the capacitive peak, was plotted against the voltage of the test pulse. The resulting current-voltage plot was fitted with Boltzman function: $I = (A + (B - A))/(1 + \exp((V-V_{1/2})/V_{slope}))$ (Equation 1), where A and B are the minimum and maximum conductance (or current), $V_{1/2}$ is the voltage where conductance is (B-A)/ 2 and V_{slope} is the slope factor. The TEA dose response data was fit by function $I/I_{max} = a+((1-a)/(1+([TEA]/IC50)^{h}))$ (Equation 2), where [TEA] is the TEA concentration, IC50 is the concentration at which inhibition is half maximal, h is the slope factor (Hill coefficient) and a is the fraction of unblocked current at saturating [TEA]. Statistical significance of differences was calculated using student's t-test.