De-novo *KCNA2* mutations cause hereditary spastic paraplegia

Running head: *KCNA2* in hereditary spastic paraplegia

Andreea Manole [1,2], Roope Männikkö, PhD [1,2] , Michael G. Hanna, MD, PhD [2], SYNAPS study group, Dimitri M. Kullmann, MD, DPhil [3], Henry Houlden MD, PhD [1,2]

[1] Department of Molecular Neuroscience and Neurogenetics Laboratory, [2] MRC Centre for Neuromuscular Diseases, [3] Department of Clinical and Experimental Epilepsy, UCL Institute of Neurology, Queen Square, London WC1N 3BG, UK.

Corresponding author: Henry Houlden for the SYNAPS study group, Department of Molecular Neuroscience, UCL Institute of Queen Square, London WC1N 3BG, UK. Tel: +44 20 3448 4249 Fax: +44 20 3448 4786, Email: h.houlden@ucl.ac.uk

Number of characters in title: 59

Number of characters in running head: 38

Number of words in main text: 350

Number of references: 5

Number of figures: 1

In a recent study Helbig et al. [1] identify a recurrent variant in *KCNA2*, which encodes the voltage-gated potassium channel, Kv1.2, as a novel cause of hereditary spastic paraplegia (HSP) in two unrelated families. Previously, gain-of-function and dominant-negative mutations in *KCNA2* have been implicated in early-onset epileptic encephalopathies, ataxia or intellectual disability [2-4], making this an interesting and unexpected finding, where epilepsy and other neurodegenerative disorders overlap.

We therefore, examined whole exome data and performed Sanger sequencing in a cohort of individuals with likely inherited neurological disorders and found one heterozygous *de novo* case with the same mutation described by Helbig et al. [1] (Figure 1). Their third family had a KCNA2 *de novo* mutation, however, had ataxia rather than spasticity at 20 years.

The proband reported here presented as a teenager with progressive walking problems. There was no history of seizures or other neurological features. Clinical examination revealed dysarthria, spastic paraplegia and mild ataxia. The *KCNA2* mutation we identified is a substitution of the first arginine residue in the voltage-sensing S4 segment of Kv1.2 with a histidine residue (c.881G>A, p.R294H). We performed site-directed mutagenesis on the complementary DNA of *KCNA2*, injected the RNA into *Xenopus laevis* oocytes and recorded currents using two-electrode voltage clamp. The current amplitude of the R294H mutant channel was reduced compared to wild type (I_{max}(Kv1.2)=5.7±0.6µA, n=15, I_{max}(R294H)=2.2±0.4µA, n=12, $p<0.001$, student's t-test) and the voltage dependence of activation was right shifted ($V_{1/2}$(Kv1.2)=-19.7±2.1mV, n=15, $V_{1/2}$(R294H)=-11.2±1.5mV, n=12, $p<0.01$, student's t-test). Mutation R294H also exerted dominant negative effects when co-expressed with wild type (Figure 1).

To conclude, we have identified a patient with HSP carrying a *de novo* R294H mutation in Kv1.2. Our results confirm that the R294H mutation is pathogenic, exerting a loss-of-function effect, consistent with published data [1]. The discovery of *KCNA2* mutations in epilepsy, ataxia and HSP extends the phenotypes that can be associated with this gene but also highlights the clinical importance of the position of the mutated amino acid residue [5]. Further studies are required to better understand the molecular basis of this novel phenotype and how the specific mutation leads to spastic paraplegia.

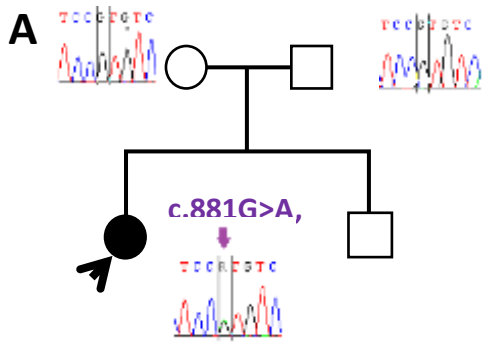
Potential Conflicts of Interest and Funding

The authors have no conflict of interest. The authors would like to thank the participants of the study and the SYNAPS study group for their essential help with this work. This study was supported by the Medical Research Council (MRC UK), The Wellcome Trust (Synaptopathies strategic award (104033) and The NIHR UCL/UCLH Biomedical Research Centre (BRC).

References

1. Helbig, K.L., et al., *A recurrent mutation in KCNA2 as a novel cause of hereditary spastic paraplegia and ataxia.* Ann Neurol, 2016. **80**(4).
2. Syrbe, S., et al., *De novo loss- or gain-of-function mutations in KCNA2 cause epileptic encephalopathy.* Nat Genet, 2015. **47**(4): p. 393-9.
3. Pena, S.D. and R.L. Coimbra, *Ataxia and myoclonic epilepsy due to a heterozygous new mutation in KCNA2: proposal for a new channelopathy.* Clin Genet, 2015. **87**(2): p. e1-3.
4. Hundallah, K., et al., *Severe early-onset epileptic encephalopathy due to mutations in the KCNA2 gene: Expansion of the genotypic and phenotypic spectrum.* Eur J Paediatr Neurol, 2016. **20**(4): p. 657-60.
5. Kara, E., et al., *Genetic and phenotypic characterization of complex hereditary spastic paraplegia.* Brain, 2016. **139**(Pt 7): p. 1904-18.

Figure 1.A) Pedigree of the KCNA2 family. Open symbols represent unaffected individuals and filled symbols represent the affected individual. The proband is indicated by an arrow. Sanger sequencing chromatograms surround the symbols and show segregation of mutations. Black bars encompass the sites of interest. **B)** Structural conservation of the mutated amino acid residues in Kv1.2 across 6 species (single letters = amino acid residues; black = identical; grey = conserved substitution; blue = loss-of-function mutations [2]; red = gain-of-function mutations [2]; purple = mutation found in this study); conservation among species of the affected amino acid residues was determined using Ensembl to retrieve the sequences and ClustalW2 software for multiple sequence alignment. **C)** Representative current traces of wild type and R294H Kv1.2 channels recorded in Xenopus laevis oocytes using two electrode voltage clamp. Test voltages ranged from -150 to +90 mV in 10 mV increments, tail voltage was -30 mV. Capacitive transients are not shown. The dashed lines show zero current level. The scale bars are 50 ms and 2 µA. **D)** The tail current amplitude is plotted against the test voltage for wild type (solid circles), n=15, R294H (solid squares), n=12, and heteromeric wild type/R294H channels (open triangles), n=16. Data shows the mean ± standard error of mean. The dashed lines show zero current level.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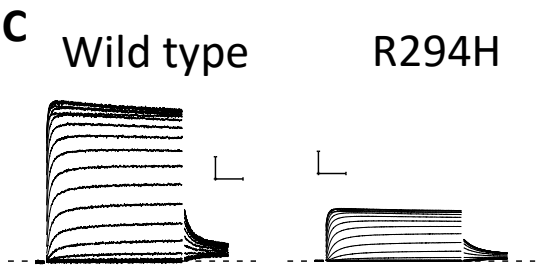
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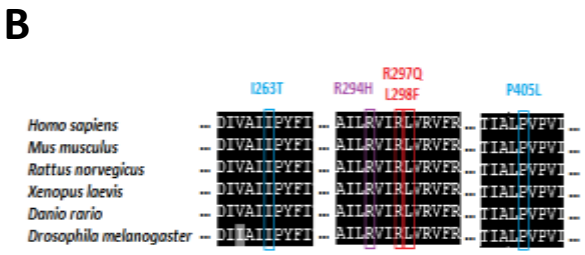
Wild type
R294H


B
I263T
R294H
R297Q
L298F
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Homo sapiens
Mus musculus
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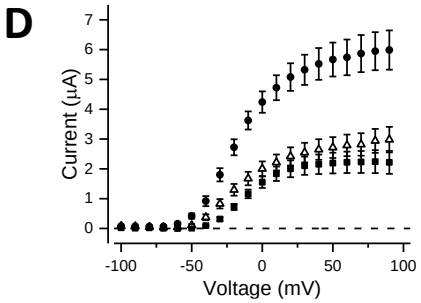
Danio rario
Drosophila melanogaster


D
Current (μA)
Voltage (mV)
-100
-50
0
50
100
0
1
2
3
4
5
6
7