



## Clinical Exome | Genome Reports

# Ataxia and myoclonic epilepsy due to a heterozygous new mutation in *KCNA2*: proposal for a new channelopathy

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### ABSTRACT

We have recently performed exome analysis in a 7 year boy who presented in infancy with an encephalopathy characterized by ataxia and myoclonic epilepsy. Parents were not consanguineous and there was no family history of the disease. Exome analysis did not show any pathogenic variants in genes known to be associated with seizures and/or ataxia in children, including all known human channelopathies. However, we have identified a mutation in *KCNA2* that we believe to be responsible for the disease in our patient. This gene, which encodes a member of the potassium channel, voltage-gated, shaker-related subfamily, has not been previously described as a cause of disease in humans, but mutations of the orthologous gene in mice (*Kcna2*) are known to cause both ataxia and convulsions. The mutation is c.890C>A, leading to the amino acid substitution p.Arg297Gln, which involves the second of the critical arginines in the S4 voltage sensor. This mutation is characterized as pathogenic by five different prediction programs. RFLP analysis and Sanger sequencing confirmed the presence of the mutation in the patient, but not in his parents, characterizing it as *de novo*. We believe that this discovery characterizes a new channelopathy.

### CLINICAL CASE REPORT

Clinical data can be accessed at <https://phenomecentral.org/P0000785>. The patient evolved well until, at 15 months of age, had a first febrile seizure, soon followed by a non-febrile seizure. Neurological examination at 17 months showed hypotonia and mild ataxia (he had just started to walk). There was no dysmorphism. The Computerized axial tomography (CAT) scan was normal, but electrocardiogram (EEG) showed slow basal rhythm. Afterwards there was worsening

of the seizures, frequently after respiratory infections, aggravation of the ataxia and obvious delay of development. After the age 2 years he started presenting myoclonic convulsions, absence crises and the EEG pattern changed to spike-slow wave complexes (3 cycles/s). Progressively there was clinical deterioration with aggravation of the ataxia and tonic-clonic convulsions. Brain magnetic resonance imaging (MRI) remained normal, but the EEG became more disorganized with paroxysms of spike-irregular slow waves at 2–2.5 cycles/s. In spite of treatment with ethosuximide, topiramate and clobazam the clinical symptoms were not properly controlled, with development of tremor of the extremities, loss of sphincter control and hyperkinetic behavior. At this point (age 7), exome analysis was performed.

### EXOME REPORT

Exome sequencing was performed at the Centre for Applied Genomics, The Hospital for Sick Children, Toronto, Canada, using the TargetSeq Exome kit V2 and an Ion Torrent Proton platform (Life Technologies, Carlsbad, CA). Signal processing, base calling, mapping to reference genome and variant calling were performed using the Ion Torrent Suite version 4.0 (Life Technologies) following the Ion Torrent Proton TargetSeq pipeline using default parameters. There were 14,772,977,596 base reads, of which 7,661,517,157 (51.9%) were on target. There were 46,208,266 bases in target regions, with an average base coverage depth of  $\times 165.8$ . Of the target bases, 94.2% were covered at  $\times 20$  and 70.1% were covered at  $\times 100$ .

Bioinformatics analysis was performed using several softwares, but especially ENLIS Genome Research (Enlis, LLC,

Berkeley, CA) and Mendel, MD a program for exome analysis developed in-house (RGCCCL Cardenas and SDJ Pena, in preparation). We observed a total of 49,156 genetic variants, with 30,771 variants in the coding regions of structural genes, distributed as follows: 14,194 missense, 647 frameshift, 110 nonsense and nonstop, 29 mistart/mistop, 231 inframe indels and 15,560 synonymous.

## RESULTS AND DISCUSSION

We performed a phenotype-based search by entering the two mammalian phenotype ontology terms Ataxia (MP:0001393) and Epilepsy (MP:0002064) in the Human-Mouse: Disease Connection (<http://www.informatics.jax.org/mgihome/homepages/humanDisease.shtml>). The result was a list of 630 human genes that corresponded to the specified phenotypes in humans and/or mice. We then ran the patient's VCF data (the VCF file is enclosed in the Supporting Information as Appendix S1) in against the short list of 630 genes using the Mendel, MD software, filtering out the variants using the simple categories: present in dbSNP Build 129, Read Depth < 10, frequency > 0.005. From this filter process, which minimized the chance of discarding any relevant variants, emerged a very short list of 23 genes: *AARS*, *ABCD2*, *ANK3*, *APC2*, *CACNA2D2*, *CNTNAP1*, *ERBB2*, *GJA1*, *GNAQ*, *HYDIN*, *KCNA2*, *KCNMA1*, *KCNQ2*, *KIF1A*, *LAMA2*, *PROM1*, *QKI*, *SHANK3*, *SLC12A6*, *TP53*, *TSC1*, *UCHL1* and *WNT1*. On the basis of the phenotype, we quickly discarded 16 of these 18 genes with clinical data listed in OMIM (*AARS*, *ANK3*, *ERBB2*, *GJA1*, *GNAQ*, *HYDIN*, *KCNMA1*, *KCNQ2*, *KIF1A*, *LAMA2*, *PROM1*, *SHANK3*, *SLC12A6*, *TP53*, *TSC1*, *UCHL1* and *WNT1*) with only two candidates persisting (*KCNQ2* and *KCNMA1*). *KCNQ2* displayed a heterozygous frameshift mutation, which was seen in the exomes of other patients with quite different phenotypes, being thus classified as a false-positive. The pathogenic impact of *KCNMA1* and other missense variants (see below) was assessed using Alamut Visual 2.4 (Interactive-Biosoftware, Rouen, France). The *KCNMA1* variant had very low allelic ratio (16%) and was considered tolerated by SIFT (<http://sift.jcvi.org>) and Polyphen-2 (<http://genetics.bwh.harvard.edu/pph2>), being thus discarded from analysis.

We then turned our attention to the other six variants that did not have a pathological phenotype listed in OMIM: *ABCD2*, *APC2*, *CACNA2D2*, *CNTNAP1*, *KCNA2* and *QKI*. *CACNA2D2* was a heterozygous frameshift mutation, which was also seen in other exomes of patients with very different phenotypes, being thus classified as a false-positive. *CNTNAP1*, *APC2* and *QKI* had characteristics of benign polymorphisms according to SIFT and Polyphen-2 and/or presence of variants in patients with other phenotypes. The *ABCD2* variant had some pathogenic features, but the phenotype was of a late-onset ataxia in mice (1), rather than the infantile onset we saw in our patient.

In contrast to foregoing, we found a variant in the *KCNA2* with very strong pathogenic characteristics (c.890G>A; p.Arg297Gln): it had a Rawscore of 4.5 (PHRED = 24.2) in CADD (<http://cadd.gs.washington.edu>), it belonged to class C35 (GV: 0.00–GD: 42.81) in the Align-GVGD program (<http://agvgd.iarc.fr>), it had a deleterious (score: 0) in SIFT, it was considered disease causing (p-value: 1) by Mutation Taster (<http://www.mutationtaster.org>), and it was classified as 'Probably Damaging' by Polyphen-2.

The variant had 181 reads, 57% of which were mutant. It was confirmed by restriction fragment length polymorphism (RFLP) analysis with *MspI* and Sanger sequencing (Fig. S1) and was not present in any of the parents. It was not seen in more than 6500 normal exomes in the Exome Variant Server, NHLBI GO Exome Sequencing Project (ESP), Seattle, WA ([evs.gs.washington.edu/EVS/](http://evs.gs.washington.edu/EVS/)).

*KCNA2* encodes a member of the potassium channel, voltage-gated, shaker-related subfamily. This member contains six membrane-spanning domains. The coding region is intronless, and the gene is clustered with genes *KCNA3* and *KCNA10* on chromosome 1 ([www.genecards.org](http://www.genecards.org)). The variant found in our patient involved the second of the critical arginines in the S4 helix, which corresponds to the voltage sensor (2, 3), as shown in Fig. S2. *KCNA2* has not been previously described as a cause of disease in humans, but mutations of the orthologous gene in mice (*Kcna2*) were known to cause both ataxia [c.1205T>C (p.Ile402Thr) mutation in the S6 helix] and convulsions in *Kcna2*-null animals (4–6).

Xie et al. (5) observed that pharmacological treatment with acetazolamide was capable of partially rescuing the motor incoordination in mice with the *Pingu* mutation in *Kcna2*. Because the patient was not responding well to his medication, he was started on acetazolamide 375 mg/day. There was rapid clinical improvement in both the ataxia and seizure control. The use of ethosuccimide could be stopped and now the patient only has had seizures after physical exertion. Functional studies are under way to confirm this *KCNA2* mutation as a new human channelopathy.

## SUPPORTING INFORMATION

The following Supporting information is available for this article:

**Figure S1.** Fragment of Sanger sequencing showing that the patient is a c.890G>A heterozygote (arrow) and that both parents are normal homozygotes.

**Figure S2.** Voltage-gated K<sup>+</sup> channels are assembled from four subunits that each contains six helical transmembrane segments, numbered S1–S6. They are built from a domain that senses voltage and another domain that forms the pore. S5 and S6 make up the pore-forming domain – S6 is believed to be the inner helix, which lines much of the pore. S1–S4 surround the pore domain and S4 functions as the main voltage-sensing domain. This model [reprinted from Yi and Jan (7) with permission] shows two subunits. The pore is lined by residues in the P region and S6. The segment S4 is probably the voltage-sensor and is characterized by a series of positively charged arginine residue at every third position. The arginine mutated in the patient (p.Arg297Gln) is located in the

S4 voltage sensor (arrow). The approximate position of the mouse mutation p.Ile402Thr mutation in the S6 helix (5) is indicated with a red dot.

**Appendix S1.** VCF file.

Additional Supporting information may be found in the online version of this article.

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