

## A Recurrent Mutation in *KCNA2* as a Novel Cause of Hereditary Spastic Paraplegia and Ataxia

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The hereditary spastic paraplegias (HSPs) are heterogeneous neurodegenerative disorders with over 50 known causative genes. We identified a recurrent mutation in *KCNA2* (c.881G>A, p.R294H), encoding the voltage-gated K<sup>+</sup>-channel, K<sub>v</sub>1.2, in two unrelated families with HSP, intellectual disability (ID), and ataxia. Follow-up analysis of >2,000 patients with various neurological phenotypes identified a de novo p.R294H mutation in a proband with ataxia and ID. Two-electrode voltage-clamp recordings of *Xenopus laevis* oocytes expressing mutant K<sub>v</sub>1.2 channels showed loss of function with a dominant-negative effect. Our findings highlight the phenotypic spectrum of a recurrent *KCNA2* mutation, implicating ion channel dysfunction as a novel HSP disease mechanism.

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The hereditary spastic paraplegias (HSPs) are a heterogeneous group of neurodegenerative disorders characterized by spasticity and weakness in the lower extremities. Complicated forms may also include other neurological

manifestations, such as intellectual disability (ID), epilepsy, ataxia, polyneuropathy, optic nerve involvement, or amyotrophy.<sup>1</sup> To date, over 50 genes have been identified for HSPs, involved in axonal transport, lipid metabolism, autophagy, myelination, DNA repair, membrane trafficking and vesicle formation, cellular signaling, and protein folding.<sup>2,3</sup> Despite these discoveries, the genetic basis of approximately 40% of familial HSPs remains unknown.<sup>4</sup> Although ion channel dysfunction plays an important role in the pathogenesis of other disorders of the central nervous system, including the epilepsies<sup>5</sup> and ataxias,<sup>6,7</sup> to date ion channels have not been implicated in HSPs.

De novo gain-of-function and dominant-negative mutations in *KCNA2*, encoding the Shaker-type voltage-gated potassium channel, K<sub>v</sub>1.2, have recently been implicated in early-onset epileptic encephalopathies.<sup>8–10</sup> *KCNA2* belongs to the K<sub>v</sub>1 subfamily of voltage-gated potassium channels that plays a crucial role in the repolarizing phase of action potentials and neuronal excitability. K<sub>v</sub> channel subunits contain six transmembrane helices (S1–S6), comprising a voltage-sensing (S1–S4) and pore (S5–S6) domain. They form homomeric or heteromeric channels. K<sub>v</sub>1.2 channels are expressed in both excitatory and inhibitory neurons and are concentrated in axon initial segments and axon terminals.<sup>11,12</sup>

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Here, we report on a novel recurrent missense mutation within the K<sub>v</sub>1.2 voltage sensor associated with variable phenotypes, including hereditary spastic paraplegia, ataxia, and ID.

**Materials and Methods**

**Patients**

Family 1 was referred for diagnostic whole-exome sequencing (WES) at Ambry Genetics (Aliso Viejo, CA). Family 2 underwent WES as part of an ongoing research study investigating the genetic basis of HSP. One hundred three unrelated HSP patients previously screened for major genetic causes of HSP, including *SPAST*, *ATLI*, and *KIAA0196*, were used as a follow-up cohort. Existing trio-based WES data of approximately 1,500 patients with various neurological disorders and 500 with epilepsy were assessed as a second follow-up cohort. Informed consent was obtained for all participants and parents, where applicable. This study was approved by the local institutional review boards of the participating centers.

**Sequencing**

WES of the family quad for family 1 (proband, mother, father, and son) was performed as previously described.<sup>13</sup> Before WES, the proband from family 1 had undergone sequencing of *PLP1*, *SPAST*, and *ATLI*, deletion/duplication analysis of *PLP1*, and *SPAST* deletion analysis, which were all negative. For family 2, WES data were annotated and imported into GENESIS/GEM.app, a web-based tool for next-generation sequencing NGS data analysis.<sup>14</sup> In brief, the GENESIS/GEM.app includes the curated WES and whole-genome data from approximately 6,000 individuals/families with various neurological diseases. Candidate alterations were confirmed in available family members using automated fluorescence dideoxy sequencing. Sequencing of *KCNA2* in the follow-up cohort of 103 HSP probands was performed by automated fluorescence dideoxy sequencing.

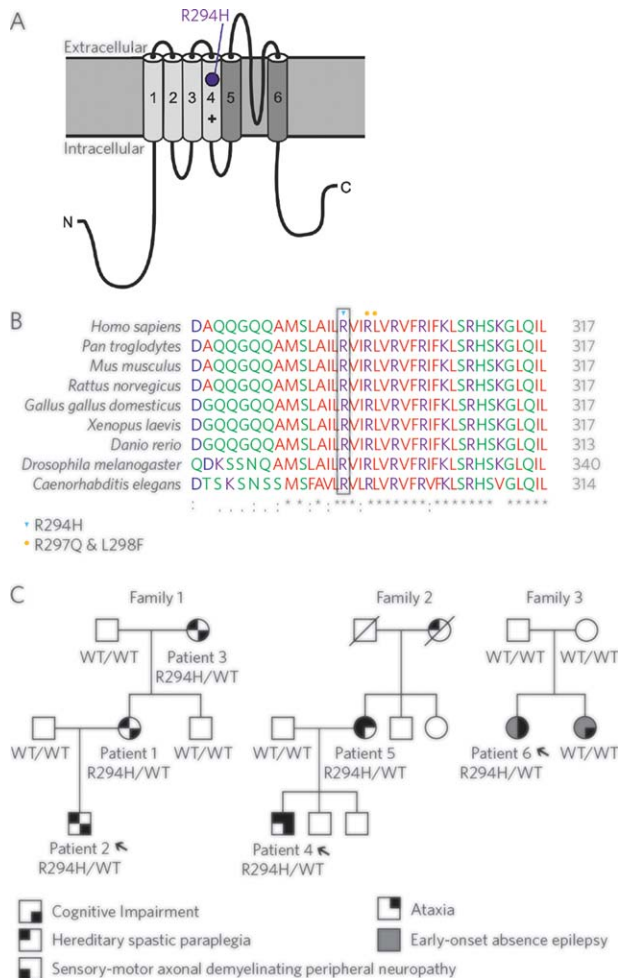
**Functional Analysis**

To engineer the mutations into human *KCNA2*, complementary (cDNA) site-directed mutagenesis was performed using Quick-Change (Agilent Technologies, Santa Clara, CA). *Xenopus laevis* oocytes were injected with mutant and/or wild-type (WT) complementary RNA (cRNA). After 2 days of incubation, potassium currents were recorded using an automated two-electrode voltage clamp system (Robocyte2; Multi Channel Systems, Reutlingen, Germany) as described previously.<sup>8</sup> Experiments were approved by the local Animal Care and Use Committee (Regierungspräsidium Tübingen, Germany).

**Results**

**Mutation Analysis**

WES in families 1 and 2 identified the c.881G>A (p.R294H) mutation, predicting substitution of a highly conserved arginine for histidine, located in the voltage sensor-forming S4 transmembrane segment of *KCNA2* (Fig 1A,B). The mutation segregates with the phenotype of spastic paraplegia in all affected individuals in an autosomal-dominant fashion (Fig 1C). The c.881G>A



**FIGURE 1: (A)** Structure of the voltage-gated potassium channel, K<sub>v</sub>1.2, with transmembrane segments S1 to S4 forming the voltage sensor domain (light gray) and segments S5 and S6 forming the pore region (dark gray) with its pore-forming loop and location of p.R294H mutation, within transmembrane segment S4, which constitutes the voltage sensor. **(B)** Evolutionary conservation of R294 amino acid residue and neighboring R297 and L298, which have been implicated in epileptic encephalopathies. **(C)** Pedigrees of all three families with identified p.R294H mutations along with cosegregation data. WT = wild type. [Color figure can be viewed in the online issue, which is available at [www.annalsofneurology.org](http://www.annalsofneurology.org).]

(p.R294H) alteration has not been reported in over 60,000 individuals in the Exome Aggregation Consortium and is predicted to be deleterious by in silico prediction models (PolyPhen and SIFT). Family 2 was the only family in the GENESIS/GEM.app data set with the p.R294H *KCNA2* mutation. Sequencing of *KCNA2* in the follow-up cohort of 103 patients with HSP did not identify any additional mutations. Screening of WES data from the second follow-up cohort of approximately 2,000 individuals with neurological disorders identified a de novo c.881G>A (p.R294H) mutation in 1 affected sister with ataxia and ID from a family with 2 siblings with early-onset absence epilepsy (Fig 1C).

**Clinical Characteristics**

Clinical features are detailed in Supplementary Table 1.

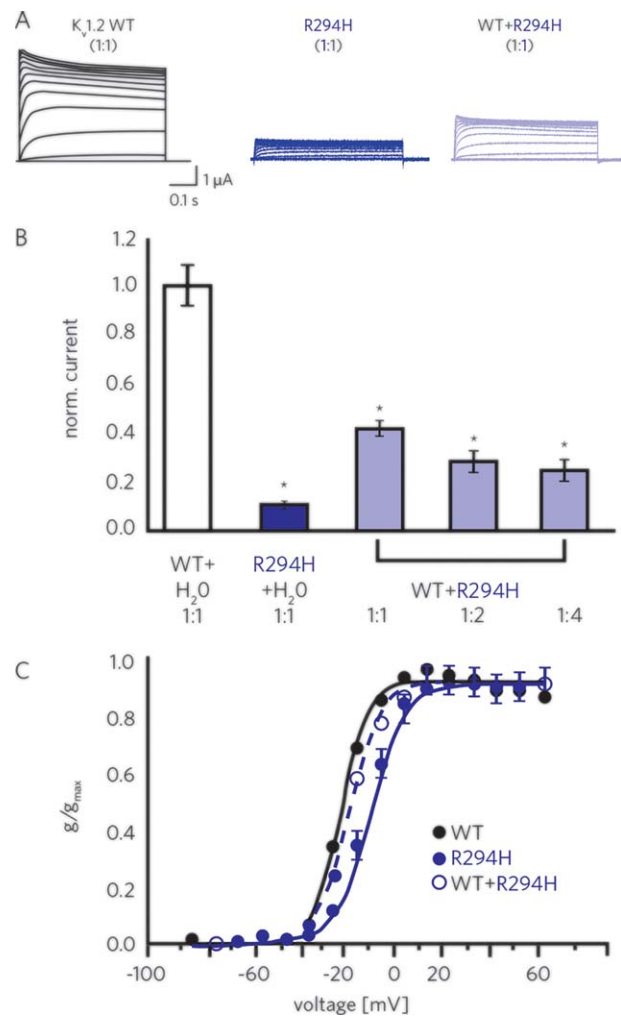
**FAMILY 1.** Family 1 consists of 3 individuals with HSP and mild cognitive deficits (Patients 1, 2, and 3). The proband (Patient 1) was a 32-year-old woman at the time of study inclusion with a clinical diagnosis of HSP, beginning around age 5 years. Her lower-limb spasticity has been progressive and she became wheelchair dependent by age 19. She had learning disabilities with slow processing speed and reported increasing impairment of fine motor skills. The proband's son (Patient 2) was 6 years old at the time of inclusion into our study and had onset of lower-limb spasticity at age 2 years, global developmental delays, and a diagnosis of autism spectrum disorder. The proband's mother (Patient 3) had mild intellectual disability and a clinical diagnosis of HSP beginning around age 5 years. Her lower-limb spasticity has been progressive and she became wheelchair dependent by age 45 years. A routine electroencephalogram (EEG) revealed focal epileptiform discharges, but no clinical seizures.

**FAMILY 2.** Family 2 consists of a mother/son pair with HSP and limb and gait ataxia (Patient 4, Patient 5). Patient 4 was 49 years old at inclusion in our study. He was diagnosed with a progressive gait disorder with frequent falls, abnormal gait pattern, and lower-limb spasticity at age 5 years. He had low normal intellect with increasing memory deficits, and a routine EEG showed focal epileptiform discharges without clinical seizures. Patient 5 had normal cognition; she had onset of spasticity at age 30 years and is able to walk independently. She also has a sensory-motor axonal demyelinating peripheral neuropathy. Both mother and son exhibit action tremor.

**FAMILY 3.** In family 3, both sisters had early-onset absence epilepsy and ID. The mutation-positive proband from family 3 (Patient 6) presented with limb and gait ataxia as well as hand tremor and fine motor difficulties at 3 years, which were not present in the mutation-negative sister. In addition, the mutation-positive sister had several prolonged febrile seizures beginning at 15 months; the mutation-negative sister only had a single febrile seizure. Spasticity was not noted in Patient 6 at age 20.

**Functional Analysis**

Current amplitudes derived from p.R294H mutant channels showed a dramatic decrease compared to those from WT (Fig 2A). Coexpression of WT and p.R294H mutant channels in different ratios with a constant amount of injected WT cRNA revealed significantly decreased current amplitudes in comparison to the same amount of WT cRNA injected alone, indicating a dominant-negative effect of mutant on WT channels (Fig 2A,B). In addition, we observed a depolarizing shift of the activation curve



**FIGURE 2: Functional effects of the p.R294H *KCNA2* mutation.** (A) Recordings of currents elicited by increasing voltage steps from a holding potential of  $-80\text{mV}$  from *Xenopus laevis* oocytes expressing either WT  $K_{v1.2}$  channels (left), R294H mutant channels (center), or both (right). (B) Amplitudes of recorded currents normalized to the mean current amplitude of the WT. Amplitudes decreased with increasing amounts of injected mutant cRNA, whereas the amount of WT cRNA remained constant, suggesting a dominant-negative effect on WT channels. Groups were statistically different (one-way ANOVA:  $p < 0.001$ ; post-hoc Dunn's method:  $p < 0.05$ ). Shown are means  $\pm$  SEM. (C) Activation curves of WT, R294H mutant, and a 1:1 expression of both clones, showing a significantly different shift to more depolarized potentials for mutant channels, consistent with a loss-of-channel function (one-way ANOVA:  $p < 0.001$ ; post-hoc Dunn's method:  $p < 0.05$ ). Shown are means  $\pm$  SEM. Lines represent Boltzmann functions fit to data points. ANOVA = analysis of variance; cRNA = complementary RNA; SEM = standard error of the mean; WT = wild type. [Color figure can be viewed in the online issue, which is available at [www.annalsofneurology.org](http://www.annalsofneurology.org).]

derived from mutant channels, consistent with a further loss of channel function (Fig 2C).

**Discussion**

We identified a recurrent missense mutation in *KCNA2* in two unrelated families with complicated HSP and in

1 individual with ataxia, tremor, and ID. Functional analysis of the p.R294H mutation revealed a dominant-negative loss of  $K_v1.2$  channel function, similar to other mutations, which we described recently outside the voltage sensor (p.I263T, p.P405L) causing epileptic encephalopathy. This is in contrast to the strong  $K_v1.2$  channel gain of function of the neighboring p.R297Q and p.L298F mutations.<sup>8</sup> The highly conserved R294 residue is the first of seven positively charged, evenly spaced gating charges in the voltage-sensing S4 segment, the movement of which causes the central  $K_v1.2$  pore to open and allow  $K^+$  ions to pass through based on their electrochemical gradient.<sup>15–17</sup> Mutations at these conserved arginine residues in the voltage-sensing domain have been demonstrated in other model systems to lead to ion leakage across the membrane and lead to a conductance, also known as omega current, which is distinct from the main pore ionic current.<sup>18,19</sup> Substitution of the first S4 arginine of the Shaker potassium channel for histidine, equivalent to the p.R294H mutation in *KCNA2*, causes an abnormal proton (omega) current at hyperpolarized potentials, which is absent in WT channels.<sup>18,20</sup>

These findings suggest that the p.R294H mutation in *KCNA2* results in loss of normal  $K_v1.2$  function and leads to a proton conduction at hyperpolarized potentials.<sup>20</sup> This may explain the neurodegenerative phenotype associated with the p.R294H mutation and account for the phenotypic difference in p.R294H mutation carriers compared to patients with (1) other mutations outside the voltage sensor (p.I263T, p.P405L) or (2) other gain-of-function voltage sensor mutations (p.R297Q, p.L298F), who have epileptic encephalopathies.<sup>8</sup>

The phenotypic spectrum of the p.R294H mutation carriers is intriguing. Two of the identified families had complicated HSP. However, the phenotype of Patient 6 from family 3 is perplexing. Patient 6 is 1 of 2 sisters with early-onset absence epilepsy, but, unlike her affected sister, had a de novo p.R294H mutation in *KCNA2* and had ataxia and tremor as the distinguishing features. This suggests that another cause of the early-absence epilepsy exists in this family. Patient 6 did not have lower-extremity spasticity at 20 years. Given the variable age of onset of spasticity in families 1 and 2, Patient 6 may not yet have developed spasticity. Alternatively, the phenotypic spectrum of the *KCNA2* p.R294H mutation may involve other movement disorders, such as ataxia, in addition to spastic paraplegia. There is some phenotypic overlap between the patients presented here and patients with other mutations in *KCNA2*,<sup>8,9</sup> in particular, cognitive impairment (families 1, 2, and 3), ataxia (families 2 and 3), impairment of fine motor skills (families 1 and 3), tremor (families 2 and 3), and prolonged febrile seizures (family 3). Although

epilepsy was not a feature of the currently described families, focal epileptiform discharges on EEG were observed in patients from all three families, suggesting an underlying susceptibility to seizures. Spasticity does not appear to be a feature in previously reported patients with *KCNA2* encephalopathy. However, a recently reported patient with epileptic encephalopathy and a de novo c.1120A>G (p.T374A) *KCNA2* mutation was found to have axial hypotonia with appendicular spasticity at 28 months, compatible with spastic quadriplegia.<sup>10</sup> Although this early-onset spastic quadriplegia is clinically distinct from the slowly progressive HSP in the families reported on here, this observation suggests that  $K_v1.2$  dysfunction in patients with epileptic encephalopathies may also lead to degeneration of the corticospinal tracts. Description of further patients with *KCNA2* mutations will delineate the full spectrum and assess whether phenotypes overlap or are mutation specific.

Ion channel dysfunction is linked to a number of disorders from cardiac arrhythmias to epilepsies. However, so far, ion channel dysfunction has not been described in the hereditary spastic paraplegias. Accordingly, identifying two unrelated HSP families with an identical *KCNA2* mutation expands the channelopathy spectrum and demonstrates a novel disease mechanism.

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### Author Contributions

K.L.H., H.L., and J.R.L. were responsible for conception and design of the study. K.L.H., U.B.S.H., D.N.S., I.K., A.C.T., J.H., J.S., A.C.C., R.H., H.M.L., W.A.A., S.T., C.J., S.L.D., L.V., K.N.K., M.S., L.S., A.E.L., R.S., I.H., H.L., and J.R.L. were responsible for acquisition and analysis of data. K.L.H., U.B.S.H., D.N.S., I.H., H.L., and J.R.L. were responsible for drafting the manuscript or figures.

### Potential Conflicts of Interest

K.L.H., D.N.S., A.C.C., R.H., H.M.L., W.A.A., and S.T. are employed by Ambry Genetics; *KCNA2* sequencing in the setting of gene panel testing and whole exome sequencing is among its commercially available tests.

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