

Reply to Gan-Or and colleagues and Manole and colleagues

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We thank Dr. Gan-Or and colleagues and Ms. Manole and colleagues for their letters and comments about the frequency and the emerging spectrum of *KCNA2* phenotypes. Gan-Or and colleagues suggest that the phenotype of the patients presented in our study represents a spectrum of the same disorder rather than a novel *KCNA2* phenotype. Manole and colleagues identify the recurrent *de novo* c.881G>A (p.R294H) mutation in *KCNA2*, which we previously reported in two unrelated families with hereditary spastic paraplegia (HSP),¹ in a further patient with spastic paraplegia and mild ataxia.

Gan-Or and colleagues did not identify pathogenic *KCNA2* variants in 158 HSP patients. This finding confirms our assessment that *KCNA2* is a rare cause of HSP and likely accounts for less than 1% of cases. HSP is genetically heterogeneous. Although *SPAST* mutations account for a large percentage of cases, mutations in other HSP-causing genes are much rarer.² Some reported HSP genes have been identified only in individual patients or families. We only identified a single family with the *KCNA2* R294H mutation in the GENESIS/GEM.app cohort and none in our confirmation cohort of 103 unrelated patients with HSP.

We would like to emphasize the distinctiveness of *KCNA2*-related HSP, further supported by the findings by Manole and colleagues. Our findings are based both on the lack of clinical overlap with *KCNA2*-encephalopathy and the unique electrophysiological features of the recurrent R294H mutation associated with HSP. Including our study and the report by Manole and colleagues, in total six affected individuals from three families carrying the R294H mutation presented with classic features of HSP, including progressive spasticity in the lower limbs. None of the individuals had seizures. All individuals with HSP carrying the R294H mutation presented with a phenotype distinct from *KCNA2*-encephalopathy, which includes early-onset refractory seizures, severe intellectual disability, and in some cases non-progressive spastic quadriplegia.^{3,4} This distinct phenotype is further supported by the

electrophysiological findings with the unique features of the HSP-related R294H mutation. Substitution of histidine for the outermost arginine of the voltage sensor in the *Shaker* potassium channel, equivalent to the *KCNA2* R294H mutation, creates a proton current through the so-called gating pore.⁵ This would clearly differentiate the underlying mechanism from previously reported *KCNA2* mutations,⁴ as discussed in our paper.¹ Our functional analysis, which has now been confirmed by Manole and colleagues in an independent experiment, identified a loss-of-function with a dominant-negative effect, but to a lesser extent than for previously reported mutations.^{1,4}

Following the discovery of a genetic disease, it is usual to see the phenotypic spectrum expand. In the epilepsies, this is well illustrated by *SCN1A* which is associated with the severe encephalopathy of Dravet syndrome and the mild disorder of GEFS+, reflecting a similarly broad epilepsy spectrum to *KCNA2*. Perhaps a closer corollary to *KCNA2* is *SLC2A1* causing glucose transporter 1 deficiency, as both genes are associated with severe and mild epilepsies, movement disorders, and in rare cases, HSP.

The phenotypic spectrum of *KCNA2* diseases is rapidly expanding. Corbett and colleagues⁶ recently reported a large family with episodic ataxia and self-limited infantile seizures evolving to mild generalized or focal epilepsies in the setting of normal intellect, a phenotype quite distinct from *KCNA2*-encephalopathy. This family carried an in-frame deletion of two amino acids in *KCNA2*, with a dominant-negative effect. These data, along with our data and those from Manole and colleagues, suggest that at least some pathogenic *KCNA2* variants have mutation-specific presentations.

AUTHOR CONTRIBUTIONS

All authors contributed equally to drafting this reply.

POTENTIAL CONFLICTS OF INTEREST

K.L.H. is 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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