PRICKLE2 Mutations Might Not Be Involved in Epilepsy

To the Editor: Previously in *The American Journal of Human Genetics*, Tao et al.[1] performed a candidate-gene analysis and reported that heterozygous mutations in *PRICKLE2* (MIM: 608501) are responsible for causing seizures in several individuals with epilepsy (MIM: 613832). Here, by exome-wide mutation screening, we found that compound-heterozygous mutations in a well-established epilepsy- and ataxia-associated gene are more likely to be the causal factor in one of the individuals reported. Our results thus suggest that *PRICKLE2* mutations should presently not be considered as causing epilepsy.

Tao et al. performed a mutation screen on a candidate gene, *PRICKLE2*, on the basis of their earlier report linking *PRICKLE1* (MIM: 608500) mutations to epilepsy[2] (MIM: 612437) and the sequence homology between *PRICKLE1* and *PRICKLE2*. We exome sequenced several individuals referred to us >10 years ago for having "atypical Unverricht Lundborg disease," i.e., early-onset myoclonus epilepsy with ataxia; samples and informed consent were obtained in accordance with an institutional review board. One sequenced individual has since been identified by one of us as the sibling of patient 4 analyzed by Tao et al., and both belong to a sibling relationship described by Bird and Shaw in 1978.[3] We think the mode of inheritance is more likely to be recessive than dominant with incomplete penetrance, given that the two siblings were affected at a young age and neither parent displayed symptoms.[3] In this individual, we identified the two heterozygous *PRICKLE2* mutations reported in his sibling by Tao et al. and determined that they were on opposite chromosomes. In addition, we identified three heterozygous mutations in *POLG* (MIM: 174763; GenBank: NM_002693.2): c.1399G>A (p.Ala467Thr), c.1491G>C (p.Gln497His), and c.2243G>C (p.Trp748Ser). We confirmed these mutations also in his sibling, patient 4 from Tao et al. Two of these variants, p.Trp748Ser and p.Ala467Thr, have been established as responsible for epilepsy and ataxia (MIM: 607459) in a recessive fashion in numerous individuals.[4–9] p.Gln497His has only been published in tandem with p.Trp748Ser,[9] suggesting co-occurrence on the same rare haplotype. We determined that c.1399G>A and c.1491G>C were on opposite chromosomes, and we inferred from this rare haplotype that one chromosome would possess the c.1399G>A mutation and that the other chromosome would possess both the c.1491G>C and c.2243G>C mutations. Importantly, the symptoms reported for those carrying these *POLG* mutations match those of the siblings, namely progressive ataxia, myoclonus, sensory neuropathy, and epilepsy. Given the >20 prior individuals with exactly the same *POLG* mutations and with this specific set of phenotypes,[5,6] the *POLG* mutations are far more likely than the postulated *PRICKLE2* variants to have caused epilepsy in this family.

Tao et al. discuss two other epilepsy-affected individuals, patient 5 and patient 6, as potentially having a heterozygous mutation and a heterozygous deletion in *PRICKLE2*, respectively. Lacking DNA samples for these two individuals, we could not ascertain whether exome-wide variant screening might reveal that they too could carry mutations in *POLG* or other more established epilepsy-related genes. However, circumstantial evidence can be obtained from large-scale variant databases. The mutation reported in patient 5, c.1813G>T (p.Val605Phe), appears twice in the Exome Aggregation Consortium (ExAC) Browser, which was not available at the time of publication of Tao et al. ExAC reports variants from over 60,000 individuals while excluding individuals with severe pediatric diseases. It now contains more than 800 carriers of missense mutations in *PRICKLE2*, including four carriers of loss-of-function variants. Although not definitive, this carrier rate seems inconsistent with a gene with dominant mutations causing a pediatric disorder.

In patient 6, Tao et al. proposed that a large heterozygous deletion is responsible for epilepsy and autism. Other papers have confirmed that large heterozygous deletions, including *PRICKLE2*, on chromosome 3 are associated with autism,[10] but nothing about epilepsy was mentioned in that or other publications.

Tao et al. followed up on the mutations with zebrafish assays, but these were not consistent, hence not conclusive, because p.[Arg148His;Val153Ile] showed more convergent-extension (CE) defects in zebrafish than the wild-type, whereas p.Val605Phe showed fewer CE defects than the wild-type. The mouse models presented for *PRICKLE2* demonstrate that knockout mice are more susceptible to seizures, but knockout models do not address whether specific missense mutations are pathological or not.

In summary, we present evidence that the reported *PRICKLE2* mutations in patient 4 are unlikely to cause epilepsy and identify *POLG* mutations that most likely caused the symptoms. These results call into question the other reported missense mutation proposed to implicate *PRICKLE2* in epilepsy. Our revised interpretation will help avoid potential misdiagnosis or other associated actions, such as family-planning decisions, given that the published mutations are often automatically considered pathological because of their annotation in public mutation databases for inherited diseases. Lastly, our experience underscores the importance of continued re-interpretation

in a time of constantly improving technology, expanding variant databases, and changing annotation.

Erin Sandford,[1] Thomas D. Bird,[2,3,4] Jun Z. Li,[5,6] and Margit Burmeister[1,5,6,7,*]
[1]Molecular & Behavioral Neuroscience Institute, University of Michigan, Ann Arbor, MI 48109, USA; [2]Department of Neurology, University of Washington, Seattle, WA 98195, USA; [3]Department of Medicine (Medical Genetics), University of Washington, Seattle, WA 98195, USA; [4]Geriatric Research, Education, and Clinical Center, VA Puget Sound Health Care System, Seattle, WA 98101, USA; [5]Department of Human Genetics, University of Michigan, Ann Arbor, MI 48109, USA; [6]Department of Computational Medicine & Bioinformatics, University of Michigan, Ann Arbor, MI 48109, USA; [7]Department of Psychiatry, University of Michigan, Ann Arbor, MI 48109, USA
*Correspondence: margit@umich.edu

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Web Resources

The URLs for data presented herein are as follows:

ExAC Browser, http://exac.broadinstitute.org
OMIM, http://www.omim.org/
RefSeq, http://www.ncbi.nlm.nih.gov/refseq/

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