

A NEW, PROGRESSIVE MYOCLONIC EPILEPSY: IS IT A CHRONICLE OF THE NONCANONICAL OR A FAILURE TO REST?

A Homozygous Mutation in Human PRICKLE1 Causes an Autosomal-Recessive Progressive Myoclonus Epilepsy-Ataxia Syndrome. Bassuk AG, Wallace RH, Buhr A, Buller AR, Afawi Z, Shimojo M, Miyata S, Chen S, Gonzalez-Alegre P, Griesbach HL, Wu S, Nashelsky M, Vladar EK, Antic D, Ferguson PJ, Cirak S, Voit T, Scott MP, Axelrod JD, Gurnett C, Daoud AS, Kivity S, Neufeld MY, Mazarib A, Strausberg R, Walid S, Korczyn AD, Slusarski DC, Berkovic SF, El-Shanti HI. *Am J Hum Genet* 2008;83(5):572–581. Progressive myoclonus epilepsy (PME) is a syndrome characterized by myoclonic seizures (lightning-like jerks), generalized convulsive seizures, and varying degrees of neurological decline, especially ataxia and dementia. Previously, we characterized three pedigrees of individuals with PME and ataxia, where either clinical features or linkage mapping excluded known PME loci. This report identifies a mutation in *PRICKLE1* (also known as *RILP* for REST/NRSF interacting LIM domain protein) in all three of these pedigrees. The identified *PRICKLE1* mutation blocks the PRICKLE1 and REST interaction *in vitro* and disrupts the normal function of PRICKLE1 in an *in vivo* zebrafish overexpression system. PRICKLE1 is expressed in brain regions implicated in epilepsy and ataxia in mice and humans, and, to our knowledge, is the first molecule in the noncanonical WNT signaling pathway to be directly implicated in human epilepsy.

COMMENTARY

Attendees of the National Institute of Neurological Disorders and Stroke's "Curing Epilepsy 2007" conference established benchmarks to help guide future epilepsy research. Three of these included: 1) the identification of unrecognized causes of epilepsy, 2) the identification of underlying mechanisms of epileptogenesis, and 3) the characterization of comorbidities in people with epilepsy. Further studies of progressive myoclonic epilepsy (PME) syndromes—diseases that confer motor and oftentimes cognitive degeneration—will undoubtedly allow epilepsy researchers to make inroads in all three of these benchmarks. PME syndromes transmit by monogenic inheritance, and thus, the sequencing of the known candidate genes in appropriate patients will help to identify new disease-causing mutations. More importantly, as is reported here by Bassuk et al., genetic mapping of kindreds containing large numbers of affected individuals will identify novel epilepsy genes. Once new PME mutations and genes are identified, investigators then can proceed with studying the biochemical and cellular processes affected by the mutant gene products. Finally, elucidation of these recently identified pathogenic processes will reveal the mechanisms that cause both the seizures and the comorbid motor and cognitive deficits.

In the Bassuk et al. paper, the authors extended the work of three previous papers that independently reported autosomal recessive ataxia epilepsy syndromes in three different Middle Eastern kindreds. In the current study, the authors used linkage mapping, followed by sequencing of all the coding regions and intron–exon boundaries in the linkage area. All patients in the three kindreds possessed a single nucleotide substitution that resulted in a single missense mutation, R104Q, in the so-called *PRICKLE1* gene. The authors performed careful controls, revealing that this nucleotide substitution was present neither in any of the 1,054 DNA samples obtained from diverse worldwide populations nor in DNA samples obtained from 300 Middle Eastern control individuals. In addition, the authors found no other variants in the entire *PRICKLE1* coding regions among samples provided by another 288 controls. These data strongly suggested that the *PRICKLE1* mutation causes this PME syndrome, and that *PRICKLE1* represents a novel epilepsy gene.

Before the publication of the paper by Bassuk and colleagues, many epilepsy researchers might have remained blissfully ignorant of *PRICKLE1*. Clearly, the results of this study give impetus to the elucidation of the wild-type and mutant functions of this novel epilepsy gene. To date, PRICKLE1 has been implicated in two biological functions: 1) a participant in the noncanonical WNT signaling pathway (called “noncanonical” because it acts independently of the of β -catenin transcriptional regulator proteins) and 2) a nuclear translocator receptor for the RE-1 silencing transcription (REST) factor protein.

Prickle was named by *Drosophila* developmental biologists when they discovered that mutant Prickle proteins produced flies with aberrant patterning of the cuticular hairs, sensory bristles, and eye units (1). *Drosophila* Prickle and its vertebrate ortholog, Prickle1, interact with frizzled proteins, which are G-protein-coupled receptors that mediate the *Drosophila* cell patterning and, in vertebrates, mediate a noncanonical WNT pathway, affecting changes in embryological development.

Overexpression of Prickle1 RNA in zebrafish embryos inhibits a key cellular rearrangement during gastrulation, called convergent extension (2). To test the effects of mutant Prickle1 on embryogenesis, Bassuk and colleagues injected either wild-type Prickle1 or Prickle1 (R104Q) mRNA into zebrafish embryos and found that Prickle1 (R104Q) conferred significantly smaller effects on convergent extension than wild-type Prickle1. The authors discussed that this finding demonstrated that the R104Q mutation disrupted the *in vivo* function of Prickle1. It should be emphasized that the gastrulation assay did not imply that the epilepsy associated with *PRICKLE1* (R104Q) resulted from impaired embryogenesis. In fact, the patients' clinical and radiographic course argues against macroscopic developmental changes. The R104Q mutation could cause microscopic changes in neuronal network formation, as was demonstrated by the effect of Prickle1 knockdown on neurite outgrowth in neuroblastoma cells (3). The generation of mice engineered with the Prickle1 (R104Q) mutation and postmortem histopathological analyses of affected patients will determine whether this mutation causes ultrastructural changes in neuronal development.

Apart from its role in the noncanonical WNT signaling pathway, Shimojo and Hersh independently identified Prickle 1 as a nuclear translocator protein that interacts with REST (4,5). The R104Q mutation is located in Prickle1's so-called PET domain, a motif of approximately 110 amino acids that is thought to mediate protein-protein interactions. In the present paper, Bassuk et al. demonstrated that Prickle1 (R104Q) failed to coimmunoprecipitate with REST, which suggests that a possible pathogenic effect of the *PRICKLE1* (R104Q) protein is the failure of proper REST translocation.

The consequences of the impaired *PRICKLE1* (R104Q)/REST interaction on REST targeting are in dispute. Shimojo and Hersh demonstrated that heterologous cells overexpressing wild-type Prickle1 localized both Prickle1 and REST to the nucleus, whereas cells expressing various Prickle1 mutations or with knockdown Prickle1 localized REST to the cytoplasm (4,5). In contrast, Bassuk et al. demonstrated that wild-type *PRICKLE1* colocalized with REST in the cytoplasm, but *PRICKLE1* (R104Q) colocalized with REST in the nucleus. Therefore, it is unclear whether, *in vivo*, *PRICKLE1* (R104Q) will enhance or inhibit REST's ability to enter the nucleus to repress the transcription of neuron-specific genes.

The possibility that the *PRICKLE1* (R104Q) mutation causes a degenerative epilepsy by modulating REST-mediated inhibition of gene transcription raises the question of whether or not this PME results from cell death and structural changes, or instead, from altered neuronal hyperexcitability or synaptic strength. Many of the genes modulated by REST encode ligand-gated and voltage-gated ion channels (6). Future histopathological studies of patients homozygous for *PRICKLE1* (R104Q) and electrophysiological and ultrastructural studies of mice engineered with the *prickle1* (R104Q) mutation will reveal what causes this PME. The possibility of altered synaptic strength is particularly intriguing, because it would suggest that therapeutics targeted to ligand-gated and voltage-gated ion channels may be more effective in this PME syndrome than in those associated with structural changes.

In addition to identifying the *PRICKLE1* (R104Q) mutation and testing two of its functional consequences, the Bassuk et al. study also phenotypically characterized this novel epilepsy syndrome, including its comorbidities. The patients in all three kindreds experienced a gait ataxia that developed in childhood between ages 4 and 5. The patients then developed myoclonic seizures between ages 5 and 10; the ataxia and myoclonic seizures worsened as the patients grew older. None of the patients experienced impaired cognition. These key features of progressive myoclonic seizures and ataxia in the setting of preserved cognition make this latest PME syndrome very similar to Unverricht-Lundborg disease (epilepsy progressive myoclonic 1 [EPM1]). However, while this syndrome is caused by *PRICKLE1*, EPM1 is caused by homozygous mutations in cystatin B (*CSTB*)—a cysteine protease inhibitor that is believed to prevent cell damage from lysosomal proteases. Why would mutant *PRICKLE1*, a very distinct protein from *CSTB*, produce a near phenocopy of a *CSTB* mutation? Moreover, why do *PRICKLE1* (R104Q) patients escape MRI evidence of neuronal injury, whereas EPM1 patients possess structural MRI abnormalities (7), and *ctsb* knock-out mice demonstrate neuronal atrophy and apoptosis (8,9)? It is likely that data from further study of the *PRICKLE1* (R104Q) patients and the creation of a mouse engineered with the *prickle1* (R104Q) mutation will answer these questions and thereby mark further progress toward the achievement of the epilepsy research benchmarks.

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