

THE CADHERIN SUPERFAMILY AND EPILEPTOGENESIS: END OF THE BEGINNING?

X-Linked Protocadherin 19 Mutations Cause Female-Limited Epilepsy and Cognitive Impairment. Dibbens LM, Tarpey PS, Hynes K, Bayly MA, Scheffer IE, Smith R, Bomar J, Sutton E, Vandeleur L, Shoubbridge C, Edkins S, Turner SJ, Stevens C, O'Meara S, Tofts C, Barthorpe S, Buck G, Cole J, Halliday K, Jones D, Lee R, Madison M, Mironenko T, Varian J, West S, Widaa S, Wray P, Teague J, Dicks E, Butler A, Menzies A, Jenkinson A, Shepherd R, Gusella JF, Afawi Z, Mazarib A, Neufeld MY, Kivity S, Lev D, Lerman-Sagie T, Korczyn AD, Derry CP, Sutherland GR, Friend K, Shaw M, Corbett M, Kim HG, Geschwind DH, Thomas P, Haan E, Ryan S, McKee S, Berkovic SF, Futreal PA, Stratton MR, Mulley JC, Gecz J. *Nat Genetics* 2008;40(6):776–781. Epilepsy and mental retardation limited to females (EFMR) is a disorder with an X-linked mode of inheritance and an unusual expression pattern. Disorders arising from mutations on the X chromosome are typically characterized by affected males and unaffected carrier females. In contrast, EFMR spares transmitting males and affects only carrier females. Aided by systematic resequencing of 737 X chromosome genes, we identified different protocadherin 19 (*PCDH19*) gene mutations in seven families with EFMR. Five mutations resulted in the introduction of a premature termination codon. Study of two of these demonstrated nonsense-mediated decay of *PCDH19* mRNA. The two missense mutations were predicted to affect adhesiveness of PCDH19 through impaired calcium binding. *PCDH19* is expressed in developing brains of human and mouse and is the first member of the cadherin superfamily to be directly implicated in epilepsy or mental retardation.

COMMENTARY

Because of the brain's complexity, it can be a daunting task to choose a new investigative approach to epileptogenic mechanisms. Mutations that cause epilepsy with high penetrance provide a solid starting point for laboratory investigation. Dibbens et al. offer compelling evidence that disruption of protocadherin 19, a member of the cadherin superfamily, causes epileptogenesis and developmental regression during infancy. This finding is exciting and propitious. It opens a very new angle on epileptogenesis and allows epilepsy researchers to leverage a great amount of existing knowledge of cadherin biology.

Epilepsy and mental retardation limited to females (EFMR) was first described in a single, large family in 1971 and has remained a clinical rarity. However, EFMR may be more common than previously thought. As recently suggested by Scheffer et al., the disorder's nearly unique, male-sparing pattern and varied expression in females make it easily overlooked in small families (1). Most affected females appear normal through early infancy. However, convulsions begin between

6 and 36 months (mean, 12 months) of age. Developmental regression of variable severity occurs in about half of patients, either coincidentally with seizure onset or within 1 to 2 years after it. Initial seizures often are precipitated by febrile illness. Seizures without such provocation continue into later life. Patients experience a variety of generalized and focal seizure types; in a slight majority of cases, these eventually remit between 6 and 24 years (mean, 12 years) of age. Intellectual disability generally persists after seizure remission, however. Although brain MRIs of several patients reviewed by Scheffer et al. showed no structural abnormality, neuropathological studies on a single patient treated surgically for focal seizures revealed cortical dysplasia (2). Penetrance is high: of 68 mutation-bearing females now identified by Dibbens et al., only two were classified as clinically unaffected. Male carriers of the *EFMR* gene, though spared epilepsy and significant cognitive disability, may have obsessive personality traits (1). Linkage analysis narrowed the EFMR locus to an approximately 34-megabase region of the X chromosome (1,2). However, this interval still contained several hundred genes.

Rather than taking the usual approach of further chromosomal mapping and sequencing of individual candidate genes, Dibbens et al. used "resequencing," a rapidly evolving

combination of computerized data analysis (i.e., informatics) and fast, low-cost DNA sequencing technology, to identify protocadherin 19 as the gene mutated in EFMR (3). First, the authors used the Sanger Institute's vertebrate genome annotation (Vega) database to identify all genes near the EFMR locus (4). They next performed automated sequencing on 737 genes from the probands of three EFMR families. This impressive effort revealed only a single base substitution mutation in protocadherin 19 from one patient! Although the other probands' protocadherin 19 sequences initially appeared completely normal, further bioinformatic sleuthing indicated that the protocadherin 19 sequence, as listed in the Vega database, might lack portions of the gene's functionally essential first exon. Sequencing the DNA of the predicted complete first exon revealed protocadherin 19 mutations in affected females and carrier males from all seven families studied. Thus, iron-clad genetic evidence now links protocadherin 19 to a form of epilepsy with a distinctive and carefully analyzed developmental profile.

Protocadherin 19 has not been well studied but shares basic structural and functional features with other cadherins (5–7), including a molecular plan consisting of an extracellular domain that mediates cell–cell interactions, a short segment crossing the plasma membrane, and a cytoplasmic domain that anchors to the cytoskeleton and integrates with intracellular signal transduction pathways. In the best-studied cadherins (called the classical cadherins), the extracellular domain extends from the membrane and displays a combination of protruding bumps and hydrophobic pockets along one surface. These bumps and pockets can interlock in an antiparallel zipper-like fashion with an identical cadherin on an adjoining cell. Such cadherin-based adhesion is present at adherens junctions of epithelia, between fasciculated axons, and across synapses. Furthermore, there are multiple related cadherins, each of which adheres preferentially to their own type, thus providing a mechanism for cell sorting and morphogenesis during development. Finally, the stability of classical cadherin adhesion depends on the extracellular Ca^{2+} ion concentration, and changes in cadherin binding have been implicated as activity-dependent regulators of synaptic shape and efficacy.

The cadherins are an extremely large gene superfamily. Of the over 100 human cadherin-related genes, about 70 are members of the protocadherin subgroup. Protocadherins possess extracellular domains that are homologous to those of classical cadherins and novel intracellular domains. Although the name protocadherin implies an early evolutionary origin to these genes, the opposite is true. Protocadherins are absent in invertebrates, such as flies and worms, and only began to appear in basal chordates, the immediate predecessors of vertebrates. A spectacular expansion in protocadherin gene diversity occurred in the earliest vertebrates. All the protocadherin genes are predominantly expressed in the brain. Indeed, it has been suggested

that this protocadherin expansion may be essential for the elaborate plan and sophisticated circuitry of the vertebrate brain (7). About 50 protocadherins, derived from a single ancestor, have been maintained throughout vertebrate evolution as an extraordinarily large gene cluster (8). These clustered protocadherins, located on chromosome 5 in humans and chromosome 18 in mice, are divided into α , β , and γ subgroups. The 20 or so remaining protocadherins, notably including protocadherin 19, are distributed among the other chromosomes and thus are termed nonclustered or δ protocadherins.

Although protocadherins have been subjected to relatively few functional studies, this work already has revealed significant differences compared with the classical cadherins. The extracellular domains of some α and γ protocadherin seem to lack the ability to form strong adhesive bonds between neighboring cells, but instead, combine on the surface of the same cell, as heterodimeric proteins (6). In cerebellar Purkinje cells, individual cells express distinct combinations of such α and γ protocadherin pairs, which may provide a combinatorial bar code capable of uniquely identifying cell subclasses (6). Consistent with that idea, a number of protocadherin proteins have been localized to synapses using immunohistochemistry (9). Gene knock-out studies show that different protocadherins are required for normal axon tract formation by particular cell types, including protocadherin 7 for retinal ganglion cells and protocadherin 10 for striatal and thalamocortical neurons (10,11). After knockout of the entire γ protocadherin cluster, spinal motoneurons are born, migrate, extend axons, and begin to form synapses and then undergo dramatic apoptosis. If apoptotic paths are blocked, the neurons survive, but mice lack spinal cord synapses and are nearly immobile (12). Thus, protocadherins appear quite important for circuits and synapses.

By linking protocadherin 19 and EFMR in a convincing manner, Scheffer et al. (1) and Dibbens et al. have provided both a solid rationale for focused mechanistic studies and a new class of candidates for genetic screening. Future experiments will likely include an analysis of the extracellular and intracellular protein–protein interactions, cell-type and developmental stage-specific expression, and neuronal subcellular localization of protocadherin 19 and its closest cadherin family relatives. Such work may illuminate the basis for the curious male-sparing pattern in EFMR and reveal how generally important cadherin superfamily disruptions are for human epileptogenesis and cognition dysfunction.

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