Mutations in STX1B, Encoding a Presynaptic Protein, Cause Fever-Associated Epilepsy Syndromes.

Schubert J, Siekierska A, Langlois M, May P, Huneau C, Becker F, Muhl H, Suls A, Lemke JR, de Kovel CG, Thiele H, Konrad K, Kawai A, Toliat MR, Sander T, Rüschendorf F, Caliebe A, Nagel I, Kohl B, Kecskés A, Jacmin M, Hardies K, Weckhuysen S, Riesch E, Dorn T, Brilstra EH, Baulac S, Moller RS, Hjalgrim H, Koelleman BP; EuroEPINOMICS RES Consortium, Jurkat-Rott K, Lehman-Horn F, Roach JC, Glusman G, Hood L, Galas DJ, Martin B, de Witte PA, Biskup S, De Jonghe P, Helbig I, Balling R, Nürnberg P, Crawford AD, Esquerra CV, Weber YG, Lerche H. Nat Genet 2014;46:1327–1332.

Febrile seizures affect 2–4% of all children and have a strong genetic component. Recurrent mutations in three main genes (SCN1A, SCN1B and GABRG2) have been identified that cause febrile seizures with or without epilepsy. Here we report the identification of mutations in STX1B, encoding syntaxin-1B, that are associated with both febrile seizures and epilepsy. Whole-exome sequencing in independent large pedigrees identified cosegregating STX1B mutations predicted to cause an early truncation or an in-frame insertion or deletion. Three additional nonsense or missense mutations and a de novo microdeletion encompassing STX1B were then identified in 449 familial or sporadic cases. Video and local field potential analyses of zebrafish larvae with antisense knockdown of STX1B showed seizure-like behavior and epileptiform discharges that were highly sensitive to increased temperature. Wild-type human syntaxin-1B but not a mutated protein rescued the effects of STX1B knockdown in zebrafish. Our results thus implicate STX1B and the presynaptic release machinery in fever-associated epilepsy syndromes.

Synaptopathies Heat Up: Mutations in STX1B in Fever-Associated Epilepsies

Commentary

STX1B encodes syntaxin1b, a critical component of the soluble N-ethylmaleimide-sensitive factor attachment receptor (SNARE) complex that tethers synaptic vesicles at the presynaptic membrane and mediates neurotransmitter release at the synapse. Deletion of Stx1b in mice results in severe seizures and premature lethality coincident with dysfunction of neurotransmitter release at glutamatergic and GABAergic synapses. Similarly, a temperature-sensitive paralytic mutation in Drosophila syntaxin results in failure of synaptic transmission at elevated temperatures. Mutations of other genes that encode SNARE complex proteins have previously been implicated in human epilepsy, including STXBP1 (syntaxin binding protein 1), DNM1 (dynamin 1), and SNAP25 (synaptosomal-associated protein 25).

In the current study, the authors lay out a convincing case for mutation of STX1B causing fever-associated epilepsy, with evidence from both genetic and functional studies. First, the authors present data from two large pedigrees previously reported with linkage to chromosome 16p11.2 and 16p12-q12. However, the previous studies had not identified the causative gene mutations. Using next-generation sequencing technology, the authors advanced the genetic analysis of these families by performing whole exome sequencing to identify the candidate mutations in the linkage intervals on chromosome 16.

In the first family, there was a high degree of phenotypic variability among affected family members and a range of seizure types, including simple febrile seizures, and afebrile absence, generalized tonic–clonic, and myoclonic–astatic seizures. The authors defined a core phenotype that consisted of both febrile and afebrile seizures. They performed whole exome sequencing on three distant family members with the core phenotype and found a single, shared premature truncation mutation in STX1B. Follow-up sequencing of STX1B in additional family members demonstrated that the mutation cosegregated with the core phenotype.

The second family had a more homogeneous phenotype that consisted of febrile seizures and afebrile atonic, dyscognitive, or generalized tonic–clonic seizures between 10 months and 9 years of age. They performed whole exome sequencing on three distant family members with the core phenotype and found a single, shared premature truncation mutation in STX1B. Follow-up sequencing of STX1B in additional family members demonstrated that the mutation cosegregated with the core phenotype.

To further investigate the contribution of STX1B mutation to fever-associated epilepsies, they examined additional cohorts of familial and sporadic cases. The first cohort included 299 independent cases from which a single STX1B nonsense
mutation was identified in an individual with febrile seizures and epilepsy. In a second cohort of 81 adult cases of various types of epilepsy with intellectual disability, they found a single missense **STX1B** mutation (Val216Glu). Lastly, they examined a cohort of 68 parent–child trios with fever-associated epileptic encephalopathies that had undergone whole exome sequencing. In this cohort, they identified a single de novo missense mutation of **STX1B** (Gly226Arg). The two missense mutations, Val216Glu and Gly226Arg, resulted in nonconservative amino acid substitutions within the highly conserved SNARE motif region of **STX1B**.

To determine the functional consequences of the **STX1B** mutations, the authors performed a series of studies in zebrafish. First, they modeled the effect of loss-of-function mutations using morpholino antisense knockdown. Knockdown of **stxb1** to approximately 50% of wild-type levels resulted in abnormal episodic behavior and paroxysmal epileptiform events, including multispike bursts, polyspikes, and high-frequency oscillations. Elevation of body temperature resulted in increased frequency and duration of high-frequency oscillations compared to controls, and a higher occurrence of high frequency oscillations compared to baseline temperature. This unmasking of a worsened phenotype at elevated temperature supports the contribution of **STX1B** to fever-associated epilepsy.

In a final functional study, the authors performed an elegant experiment to demonstrate pathogenicity of the Val216Glu missense mutation. First, they demonstrated that the morpholino knockdown phenotype could be rescued by transgenic expression of human wild-type **STX1B**, as evidenced by reduction in seizure-like behavior and epileptiform activity. Conversely, the morpholino knockdown phenotype was not rescued by transgenic expression of human **STX1B**:Val216Glu, confirming pathogenicity of the Val216Glu mutation and suggesting that the mechanism was likely loss-of-function since the Val216Glu phenotype was indistinguishable from the knockdown.

Although the precise mechanism by which loss of **STX1B** function results in seizures has yet to be defined, it is likely to result in dysfunctional synaptic transmission. Studies from homozygous **Stxb1** null mice demonstrated a lower frequency of spontaneous release and a reduced number of docked synaptic vesicles at GABAergic and glutamatergic synapses. Genetic epilepsies can result from mutation of several genes that encode SNARE complex proteins, including **STXBP1**, **DNM1**, **SNAP25**, and now **STX1B**, leading the authors to propose this class of mutations as “synaptopathies.” The convergence of mutations in genes encoding the synaptic vesicle release machinery suggests that this is a significant pathogenic pathway and that other associated genes may also contribute to epilepsy.

by Jennifer A. Kearney, PhD

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