

EPILEPSY: IT'S NO SYN

Identification of a Mutation in Synapsin I, a Synaptic Vesicle Protein, in a Family with Epilepsy

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A four-generation family is described in which some men of normal intelligence have epilepsy and others have various combinations of epilepsy, learning difficulties, macrocephaly, and aggressive behavior. As the phenotype in this family is distinct from other X-linked recessive disorders, linkage studies were carried out. Linkage analysis was done by using X chromosome microsatellite polymorphisms to define the interval containing the causative gene. Genes from within the region were considered possible candidates and one of these, *SYN1*, was screened for mutations by direct DNA sequencing of amplified products. Microsatellite analysis showed that the region between *MAOB* (Xp11.3) and *DXS1275* (Xq12) segregated with the disease. Two-point linkage analysis demonstrated linkage with *DXS1039*, lod score 4.06 at $\theta = 0$, and *DXS991*, 3.63 at $\theta = 0$. Candidate gene analysis led to identification of a nonsense mutation in the gene encoding synapsin I that was present in all affected family members and female carriers and was not present in 287 control chromosomes. Synapsin I is a synaptic vesicle-associated protein involved in the regulation of synaptogenesis and neurotransmitter release. The *SYN1* nonsense mutation that was identified is the likely cause of the phenotype in this family.

COMMENTARY

A recent article by Garcia et al. describes a family with epilepsy, variable learning disabilities, and behavior disorders. Even though clear clinical variation exists in this family, the authors suspected this was a novel genetic syndrome because of an X-linked inheritance pattern. The discovery of a mutation in synapsin I (*SYN1*) established it as a newly described, single-gene disorder, with epilepsy as the major clinical feature. This

finding is the first example of a disorder associated with disruption of a synaptic vesicle protein, providing insight into the normal function of synapsins and their involvement in epilepsy.

All affected members of the family were male, with no male–male transmission, which suggests X-linked inheritance. This assumption was confirmed by mapping the gene to the centromeric region of the X chromosome and the subsequent identification of a mutation in *SYN1*. The mutation in *SYN1* introduces a premature stop codon (nonsense mutation) in the middle of the protein (Trp356Stop). Synapsin I is a neuronal-specific protein, and no tissue was available to confirm the effect of the mutation on the protein produced. It is likely, however, that either the messenger RNA (mRNA) is degraded (nonsense-mediated decay of mRNA transcripts is well documented) or that the truncated protein is nonfunctional. Therefore affected male patients have no synapsin I protein.

Members of the family in which the *SYN1* mutation was identified had variable phenotypes, including macrocephaly, learning disability, aggressive behavior, and epilepsy. Eight of ten affected family members had epilepsy, and one of these eight also had mild learning difficulties. The type of seizure was variable and included generalized tonic–clonic seizures, with wide-ranging onset and offset ages, as well as complex partial seizures. Two additional family members, who did not have epilepsy, had moderate learning difficulties and were macrocephalic. All 10 affected members of the family carried the *SYN1* mutation. Clinical heterogeneity is common in inherited epilepsies. The reason for the variable phenotypes in the family members may be because synapses are highly plastic, and subtle differences in the cell environment may determine the impact of loss of synapsin I.

Synapsins are a family of proteins associated with synaptic vesicles at presynaptic terminals, where they participate in phosphorylation-mediated release of neurotransmitters. Synapsin I plays a role in the regulation of synaptic formation (synaptogenesis) during neuronal development and also regulates synaptic function in the adult brain. Synapsin I is an abundant protein and is used widely as a marker of presynaptic terminals, enhanced synaptic activity, and synaptogenesis. At first one might imagine that complete loss of this synaptic vesicle protein would result in a more severe phenotype, but lessons from the knockout mouse model suggest otherwise. Mice lacking synapsin I are outwardly normal with no apparent changes in well-being or gross nervous system function (1). Thus synapsin

I is not essential for neurotransmitter release. However, mice lacking synapsin do have a reduced seizure threshold when exposed to electric current (2), and behavioral testing revealed they also have impaired learning ability (3). Examination of hippocampal neurons from the mice showed both a delay in synapse formation and defects in synaptic function.

As mentioned, synapsin I regulates both neuronal development and synaptic function. The question remains whether epilepsy is due to early disruption of neuronal development or to later abnormal regulation of synaptic function? The answer is, probably, both. The reduced rate of synaptic vesicle formation seen in mice lacking synapsin I may result in an overall shift in balance toward hyperexcitability, particularly if the release of inhibitory neurotransmitters is reduced. This theory is supported by the finding that the *SYN1* knockout mice show a decrease in release of γ -aminobutyric acid (GABA) (2). Therefore the absence of synapsin I in the mature brain could underlie the increased excitability. This increased excitability due to reduced release of GABA would explain the seizures, but what of the learning disability? Mice lacking synapsin I have delayed synapse formation in hippocampal neurons (1) supporting the possibility of abnormal development of synaptic connectivity. The slowing of synapse formation during development could account for the learning difficulties.

Families, such as the one described by Garcia et al., provide unique opportunities to identify novel epilepsy genes. With the molecular resources now available, more and more epilepsy

genes are being identified every year. Whereas many of the genes identified code for ion channels, others have a wide range of functions. The addition of *SYN1* to the list of known epilepsy genes makes the picture even more complex, with many pathways and systems involved. As more epilepsy genes are uncovered, a better understanding of the mechanisms that cause seizures will become clear. One thing is clear, epilepsy can be caused by mutations in many different genes, any of which can shift the balance between inhibitory and excitatory synaptic transmission in favor of hyperexcitability.

by Robyn Wallace, Ph.D.

References

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