Epilepsy genetics: **An abundance of riches for biologists**

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Twenty-five genes have been identified in which mutations cause epileptic seizures in mice. The gene for a Na^+/H^+ exchanger has recently been found to underlie the spontaneous mutant *slow wave epilepsy*. Studies of such mutants should help elucidate the mechanisms that control neuronal excitability.

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Epilepsy, a disorder of brain function characterized by recurrent seizures, affects roughly 1% of the human population. A seizure is a fleeting change in behavior caused by disordered, synchronized firing of populations of central nervous system neurons. A seizure may be confined to localized cerebral cortical populations, propagate widely from such localized populations, or involve widespread cortical populations from the outset. The behavioral features of a seizure are determined by which populations of neurons fire synchronously. The enormous diversity of the epilepsies is evident in the more than 40 distinct forms that have been defined in humans [1]. In biblical times, epilepsy was attributed to possession by evil spirits; even now, the etiology of epilepsy remains incompletely understood. Genetic determinants have been implicated in at least 35% of individuals afflicted with epilepsy, but the identity of the mutant genes that confer susceptibility remain largely unknown.

The mouse is a more tractable species for genetic analysis than humans, and the rate of progress in elucidating the genetic basis of inherited epilepsy syndromes in mice has, in the past three years alone, been astounding. Before 1994, only one gene defect had been linked to cortical epilepsy in mice; since then, 24 further single-gene mutations have been linked to an epileptic phenotype (Table 1, the genes are mostly listed by the names of their products). Most of these epilepsy genes — 20 out of 25 — have been identified in mice in which a gene had experimentally been deleted, modified or, less commonly, overexpressed. In most instances, the epileptic phenotype came as a complete surprise to the investigators. Indeed, epilepsy is arguably the single most common neurological abnormality recognized in mice with induced mutations.

In addition to these induced mutations found to cause epilepsy, a number of spontaneous mutations have been linked to an epileptic phenotype in mice. In a number of cases, the genes affected by these spontaneous mutations have been identified by positional cloning. One example is *slow wave epilepsy* (*SWE*), a recently discovered spontaneous mutant that exhibits both ataxia and epileptic seizures. Cox *et al.* [2] have recently described the phenotype of this mutant mouse, and the results of positional cloning, which demonstrate that the responsible defect is a null mutation of the ubiquitously expressed Na^+/H^+ exchanger, NHE1. This discovery provides a powerful new tool for elucidating the molecular mechanisms controlling neuronal excitability and selective neuronal degeneration.

The mouse genes implicated in epilepsy [3,4] encode a diverse variety of proteins (Table 1), in line with the great diversity of human epilepsy syndromes. The most common category of mutations disrupt the function of chemical synapses, followed by those that affect the intrinsic excitability of neurons. It is not surprising that either type of mutation is associated with an epileptic phenotype. For example, disruption of synaptic inhibition by targeted deletion of the gene encoding the γ-amino butyric acid type A ($GABA_A$) receptor would be expected to result in increased neuronal excitability. Genes involved in determining the structural organization of the nervous system have also been linked to epilepsy, consistent with the common occurrence of epilepsy in humans with cortical dysgenesis. For many of the remaining genes listed in Table 1, including the discovery that the *SWE* gene encodes a Na^+/H^+ exchanger, the epileptic mutant phenotype undoubtedly came as a surprise to the investigators. But such unexpected results are often of considerably greater interest, and more informative, than the expected ones.

The discovery that the *SWE* gene encodes the Na^+/H^+ exchanger NHE1 opens a rich array of questions of considerable interest to biologists. NHE1 contributes to regulation of intracellular pH and cell volume, as well as mitogenic responses to growth factors [5]. NHE1 is activated when the cell is acidified, promoting return of the intracellular pH to its steady-state level. This protein is expressed in many organs of the mouse, yet the phenotype is restricted to the brain. Might other Na^+/H^+ exchangers compensate for the absence of NHE1 in other organs, or does NHE1 have only a trivial contribution to cellular homeostasis in other organs?

SWE mice exhibit ataxia (impaired motor control), presumably because of the degeneration of deep cerebellar

nuclei; neurons in the vestibular and cochlear nuclei also undergo degeneration in these mutants. Who would have thought that a deficiency of NHE1 would cause neurons to degenerate? What is the underlying mechanism? Why does the degeneration target these neurons in particular, and spare those elsewhere in the nervous system? Cox *et al.* [2] note that the three nuclei undergoing degeneration are among those with the highest basal metabolic rates [6]. As one consequence of a high basal metabolic rate — and presumably a concomitantly high rate of neuronal firing — is a reduced internal pH [7,8], Cox *et al.* [2] suggest that neurons in these nuclei may be particularly dependent upon NHE1 activity.

How does the absence of NHE1 cause seizures? The seizures exhibited by the *SWE* mutants are similar to absence, or petit mal, seizures in humans, in that they are characterized by a brief behavioral arrest in association with a distinctive electroencephalographic abnormality consisting of 3–4 per second spike-wave discharge. Moreover, the seizures in the *SWE* mouse and humans with petit mal epilepsy are both suppressed by the anticonvulsant ethosuximide. Absence seizures are caused by the reciprocal synchronized firing of neurons in the thalamus and cerebral cortex [9], circuitry that likely subserve physiologic functions of cortical synchronization during sleep. Many possible mechanisms could account for the increased excitability of these neurons, as a wide diversity of neuronal signaling mechanisms are exquisitely sensitive to pH, including ligand- and voltage-gated ion channels, intracellular signal transduction pathways and even gap junctions between neurons.

An interesting possibility suggested by Cox *et al.* [2] is that the mechanism of the increased neuronal excitability in *SWE* mice is secondary to defective NHE1 activity in the surrounding astrocytes, rather than in the neurons themselves. One consequence of this defect in astrocytes may be reduced H^+ transport to the extracellular space, and a net alkalinization of the extracellular fluid. This seems to be a plausible mechanism, because hyperventilation —which reduces arterial CO_2 content and produces an alkalosis — reliably triggers absence seizures in children with the petit mal epilepsy disorder [10].

Apart from these questions of fundamental biological interest, the discovery made by Cox *et al.* [2] may help elucidate cellular and molecular mechanisms of absence seizures in humans. The striking similarities of the seizures in mouse and man strengthen the likelihood that the defect in the mouse may somehow provide a clue to genetic defects in humans with absence seizures. Perhaps a genetic deficiency of NHE1 itself underlies some forms of absence seizures in humans. Alternatively, perhaps the NHE1 defect will shed light on a signaling cascade that culminates in increased cortical excitability, and affected

Table 1

Mouse genes linked to an epileptic phenotype.

Gene / product	Protein type	Nature of mutation	[Ref]
Synaptic function			
Neuropeptide Y	Transmitter	Targeted deletion	[11]
Synapsin 1,2	Transmitter release	Targeted deletion	[12]
GLT-1	Transmitter transporter	Targeted deletion	[13]
$GABA_A$ β3 subunit	Transmitter receptor	Targeted deletion	[14]
$GABA_A$ δ subunit	Transmitter receptor	Targeted deletion	[15]
GluRB editing mutant	Transmitter receptor	Transgene with RNA editing deficient allele	[16]
$5HT_{2c}$ receptor	Transmitter receptor	Targeted deletion	[17]
Intrinsic excitability of neuron			
mKv 1.1 (*Shaker*)	K^+ channel	Targeted deletion	[18]
G protein coupled inward rectifier (*Weaver*)	K^+ channel	Spontaneous	[19]
α_{1A} voltage sensitive Ca^{2+} channel (*Tottering/Leaner*)	Ion channel	Spontaneous non-conservative amino acid substitution	[20]
α_{1A} voltage sensitive Ca^{2+} channel (*Tottering/Leaner*)	Ion channel	Spontaneous splice donor	[20]
β subunit of Ca^{2+} channel (*Lethargic*)	Ion channel	Splice donor	[21]
Ca^{2+} signaling			
Ca^{2+} calmodulin kinase II	Protein kinase	Targeted deletion	[22]
Inositol phosphate 3 receptor	Receptor for phospholipid metabolite	Targeted deletion	[23]
Structural organization			
Growth associated protein (GAP-43)	Axonal growth cone protein	Overexpression	[24]
p35	Neuronal-specific activation of cyclin kinase (Cdk5)	Targeted deletion	[25]
Otx-1	Homeobox-containing gene	Targeted deletion	[26]
Diverse functions			
Centromere BP-B	DNA binding protein	Transgene-induced insertion	[27]
Sphingolipid activator protein	Protein implicated in lipid metabolism	Targeted deletion	[28]
Tissue nonspecific alkaline phosphatase	Enzyme defect results in reduced GABA	Targeted deletion	[29]
Slow wave epilepsy (Na^+/H^+ exchanger)	Regulator of pH	Spontaneous (non-conservative amino acid substitution)	[2]
Huntingtin	Unknown function	Transgene containing 5′ end of human Huntingtin	[30]
Protein L-isoaspartate transferase	Protein-repair enzyme	Targeted deletion	[31]
β-hexosaminidase A	Lysosomal enzyme	Targeted deletion	[32]
Jimpy	Proteolipid protein of myelin	Spontaneous splice site	[33]
Polyglutamine repeat	Unknown	Transgene containing 146 unit CAG repeat inserted in *hprt* gene	[34]

humans have some defect elsewhere in the cascade. Although genetic studies of absence seizures in humans are under way, no genetic defect has been identified as yet.

Mutant mice with phenotypes that involve seizures are providing biologists with an abundance of riches. Questions similar to those raised concerning the *SWE* mutant are also being asked about many of the other mouse mutants in which epilepsy is part of the phenotype. Answers to these questions will teach us valuable lessons about the genetic control of neuronal excitability in physiologic circumstances. One consequence of this line of investigation is also likely to be new ways of preventing, or more effectively controlling, human epilepsies.

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