Epilepsy is a common, paroxysmal, and heterogeneous neurological disorder. Many factors, including complex genetic influences, contribute to the pathogenesis of epilepsy. However, several epilepsy syndromes are caused by mutations in single genes (Table). Most epilepsy-associated genes that have been identified within the past 5 years encode ion channels. This review illustrates the progress in defining the molecular basis of inherited epilepsies and highlights conditions caused by dysfunctional ion channels.

Ion channels may be broadly classified as voltage or ligand gated, depending on whether the primary stimulus for their activity is a change in local membrane potential or a chemical messenger (eg, neurotransmitter). The role of ion channels in neuronal excitability is well established, and the identification of mutations in neuronal ion channel genes linked to inherited epilepsy emphasizes the delicate balances that maintain electrical harmony in the central nervous system. These discoveries have also revealed new approaches to diagnosis and disease categorization, with the ultimate hope of new, gene-specific therapeutics.

IDENTIFYING AND CHARACTERIZING EPILEPSY GENES

Molecular Genetic Approaches to Finding Epilepsy Genes

Many recent contributions to our knowledge regarding the molecular basis of inherited epilepsy, especially with regard to ion channel disorders, have exploited the combination of experimental paradigms from genetics and physiology (Figure). Initially, genetic linkage analysis pinpointed regions of the human genome where polymorphic markers segregate with epilepsy in large, multigenerational kindreds. The use of genetic linkage analysis has become the traditional approach to search for genes responsible for Mendelian disorders (ie, caused by a single gene), an approach called positional cloning. In positional cloning, once linkage is established with a chromosomal region that harbors the disease-causing gene, the next challenge becomes identifying all of the protein-coding segments within that region. As the entire human genome sequence is now available, this process requires only a few hours of database mining using a desktop computer. For many of the inherited epilepsies, candidate genes (ie, genes encoding proteins potentially involved in neuronal excitability) within the critical regions identified by linkage analysis were readily evident.

To make the final link between a gene and epilepsy, mutations must be discovered in affected individuals. This is typically accomplished by means of DNA sequencing or a variety of screening methods such as single-strand conformational analysis. Mutations are extremely rare DNA variants or polymorphisms that seldom occur in unaffected individuals. A variety of mutations are easily recognized by virtue of their deleterious effects on the encoded protein. Obvious mutations include premature stop codons (nonsense mutation), insertion or deletion of nucleotides that alter the reading frame (frameshift errors), and errors that disrupt signals important for splicing messenger RNA (mRNA) (splice-site mutations). However, many muta-
Characterizing the Molecular Physiology of Mutant Ion Channels in Epilepsy

The discovery of ion channel mutations associated with inherited epilepsy prompted a series of experiments designed to reveal the fundamental functional defects conferred by these gene variants. In vivo studies of human neuronal ion channels are experimentally challenging. Therefore, research in this area has exploited recombinant (cloned) ion channels that can be manipulated and studied in a controlled laboratory environment. Recombinant ion channels are DNA copies of the corresponding human mRNA that can be introduced into cells to enable functional expression of the encoded protein.

Two cell systems are commonly used for expression of recombinant ion channels: frog oocytes and cultured mammalian cells. Oocytes from the frog *Xenopus laevis* are large enough to be directly injected with synthetic mRNA derived from a recombinant ion channel. These large cells (approximately 1 mm in diameter) can then be used in simple electrophysiologic experiments (ie, 2-electrode voltage-clamp recording). *Xenopus* oocytes are easy and inexpensive to use, but expression of endogenous ion channels can interfere with the examination of recombinant proteins, and they are not cells of human origin. Another type of expression system uses cultured human or mammalian cells (eg, HEK-293 [human embryonic kidney cells]) and a more precise method of electrophysiologic analysis (patch-clamp recording). Cultured cells have specific advantages over *Xenopus* oocytes, including improved experimental control and a human or mammalian cellular environment. However, no in vitro system provides a perfect model for living brain tissue, and
therefore we should extrapolate observations made in these experimental systems to human neurophysiology with caution.

ADVANCES IN UNDERSTANDING SPECIFIC EPILEPSY SYNDROMES

The Table provides the genetic basis of specific inherited epilepsy syndromes. Among the 28 listed disorders, 19 are associated with mutations of genes encoding 11 different ion channels and 6 other genes that have less well-defined functions. There is considerable genetic heterogeneity among the inherited epilepsies. In other words, the same clinical syndrome may be caused by mutations of different genes. For example, generalized epilepsy with febrile seizures plus (GEFS+) is associated with mutations in 2 distinct voltage-gated sodium channel genes (SCN1B and SCN1A) or in the gene encoding the γ2 subunit of γ-aminobutyric acid (GABA₆) receptors (GABRG2). Mutations of another sodium channel gene (SCN2A) are associated with a very similar syndrome. Other examples of genetic heterogeneity include autosomal dominant nocturnal frontal lobe epilepsy (ADNFLE), caused by mutations in genes encoding different subunits of nicotinic acetylcholine receptors (nAChRs), and childhood absence epilepsy, caused by mutations of the CLCN2 chloride channel gene or GABRG2. These observations illustrate the genetic complexity of the inherited epilepsies and may provide valuable new information for reassessing their classification. Specific illustrations are discussed in the following subsection.

Epilepsy Associated With Voltage-Gated Potassium Channels

The syndrome of benign familial neonatal convulsions (BFNC) is a rare inherited form of idiopathic generalized epilepsy exhibiting autosomal dominant inheritance. Convulsions occur in the neonatal period and typically resolve spontaneously after a few weeks of life. In very rare cases, a seizure disorder may occur in adulthood. The syndrome of BFNC is genetically heterogeneous with iden-

Illustrations of experimental approaches used to identify and characterize epilepsy genes. A, Linkage analysis uses large, multigenerational kindreds segregating an epilepsy phenotype. The shaded pedigree symbols represent affected individuals. Pairs of vertical lines beneath each pedigree symbol represent hypothetical alleles or haplotypes (designated by different colors) at a specific chromosomal region. An asterisk marks the allele or haplotype that segregates with the disease. Circles indicate female members; squares, male. B, Chromosome 2 ideogram illustrating the location of a single epilepsy-associated gene (SCN1A) identified by linkage analysis. C, Representative DNA sequence trace demonstrating a heterozygous mutation (C/T) that illustrates an approach to mutation screening. D, Simplified model of the SCN1A voltage-gated sodium channel illustrating the use of a recombinant ion channel for functional characterization of an epilepsy-associated mutation (pink circle). E, Functional characterization of a recombinant, mutant sodium channel using cultured mammalian cells and patch-clamp recording. The DNA encoding a recombinant ion channel is introduced into cultured cells by transfection (droplets represent transfection reagent). A single cell is illustrated undergoing patch-clamp recording and resulting in a typical sodium current (INa).
tified loci on chromosomes 8q and 20q. In 1998, 2 groups identified the potassium channel gene KCNQ2 as the 20q gene and KCNQ3 as responsible for the chromosome 8q-linked syndrome.1,2 Both potassium channels exhibited a high degree of amino acid sequence identity with KCNQ1, a voltage-gated potassium channel gene previously linked to congenital long QT syndrome, an inherited cardiac arrhythmia.3 The KCNQ2 and KCNQ3 potassium channels coassemble to generate potassium channels that generate ionic currents resembling neuronal M-currents.4 Neuronal M-currents modulate excitability by dampening the tendency for repetitive firing. Neuronal M-currents are inhibited by muscarinic acetylcholine receptor agonists and activators of other types of neurotransmitter receptors. Mutations in KCNQ2 or KCNQ3 reduce function of the encoded potassium channel by a dominant-negative mechanism consistent with the autosomal dominant inheritance pattern of BFNC.5

Epilepsy Associated With Voltage-Gated Sodium Channels

In 1997, Scheffer and Berkovic6 described GEFS+, a newly recognized epilepsy syndrome with autosomal dominant inheritance. The syndrome was named in reference to the common occurrence of febrile seizures in early childhood that often persisted beyond 6 years of age. In addition to febrile seizures, affected adult members of GEFS+ families exhibited atefibre seizures and seizures with multiple clinical phenotypes. Missense mutations in SCN1A encoding a neuronal voltage-gated sodium channel α subunit account for most GEFS+ cases,7,11 but heritable defects in 2 other sodium channel genes (SCN1B and SCN2A)10,12 and a GABA receptor subunit gene (GABRG2)14,15 can also cause the disorder or clinically similar conditions.

The functional properties of mutant neuronal sodium channels associated with inherited epilepsy have been described previously.16-20 Three SCN1A mutations associated with GEFS+ exhibit defects in fast inactivation gating characterized by a persistent, nonactivating current during membrane depolarizations.17 These findings suggest that in some cases, SCN1A mutations promote a gain of function in sodium channels, leading to neuronal hyperexcitability. Other SCN1A mutations associated with GEFS+ cause other types of functional impairments that may lead to loss of function.16,22 Whether gain of sodium channel function in excitatory neurons or loss of function in inhibitory neurons is the primary mechanism responsible for epilepsy in GEFS+ remains unclear.

Severe myoclonic epilepsy of infancy is a rare convulsive disorder characterized by febrile seizures with onset during the first year of life followed by intractable epilepsy, impaired psychomotor development, and ataxia.21,22 Seizures in this disorder are usually unresponsive to anticonvulsant drugs. Recently, several heterozygous SCN1A mutations, including several instances of de novo mutations, have been reported in probands with severe myoclonic epilepsy of infancy, including missense, nonsense, and insertion/deletion alleles.23-26 The observed clinical similarities between severe myoclonic epilepsy of infancy and GEFS+, including the frequent occurrence of febrile seizures and shared molecular genetic origins, have prompted the idea that the 2 disorders represent a spectrum of severity of the same disease.27 Many SCN1A mutations associated with this disorder seem to encode nonfunctional sodium channels, leading to the suggestion that severe myoclonic epilepsy of infancy is caused by loss-of-function mutations.

Autosomal Dominant Nocturnal Frontal Lobe Epilepsy

Individuals affected with ADNFLE experience short, partial seizures during sleep. Episodes often occur in clusters, localize to the frontal lobes in ictal electroencephalographic recordings, and involve nonspecific auras along with brief motor seizures. Most cases are mild and respond well to carbamazepine treatment.

Neuronal nAChRs are pentameric complexes with variable subunit composition. The most common nAChR composition contains α, and β, subunits. Mutations in 2 genes (CHRNA4 and CHRNA2) encoding distinct nAChR subunits (α, and β,) have been associated with ADNFLE.28 A third genetic locus has been identified near a cluster of other nAChR subunit genes (15q24), but mutations linked to this form of the disease have not yet been discovered. Neuronal nAChRs are located in presynaptic membranes of the cerebral cortex, where they facilitate excitatory and inhibitory neurotransmitter release.

Functional characterization of mutant nAChR subunits in Xenopus oocytes has suggested a loss or a gain of receptor function,29 making it difficult to determine the precise molecular mechanism underlying ADNFLE. A recent study revealed that mutations in the α, and β, nAChR subunits interfere with calcium ion modulation of receptor function in a dominant manner, suggesting an alternative explanation for ADNFLE.30 Acetylcholine activation of wild-type α, and β, receptors is normally potentiated by extracellular calcium ions. In rapidly firing excitatory synapses, postsynaptic glutamate receptors deplete local extracellular calcium ions and reduce calcium potentiation of nAChRs, a possible negative feedback mechanism to limit further presynaptic glutamate release. This feedback mechanism may be disabled in presynaptic membranes expressing mutant α, and β, receptors, possibly leading to increased excitatory neurotransmitter release under some conditions such as rapid synchronous neuronal firing during sleep.30

Epilepsies Associated With Voltage- and Ligand-Gated Chloride Channels

There is a common notion that seizures occur because of imbalances between excitatory and inhibitory neuronal activity in the brain. The GABA, receptors are critical mediators of inhibitory neuronal activity. In the healthy postnatal brain, activation of GABA, receptors triggers an influx of chloride ions that render the postsynaptic membrane potential more negative (hyperpolarization). This hyperpolarization counters excitatory synaptic inputs that would otherwise depolarize the postsynaptic membrane and promote action po-
tential firing. The ability of GABA<sub>A</sub> receptors to mediate chloride influx is dependent on other factors, including potassium-chloride cotransporters and voltage-gated chloride channels that maintain a low intracellular concentration of this anion.

Mutations in 2 genes that encode subunits of GABA<sub>A</sub> receptors and a third gene that encodes a voltage-gated chloride channel have been associated with inherited epilepsy. The GABA<sub>A</sub> receptors are composed of 5 subunits encoded by multiple different gene families (α, β, γ, δ, ε, π, and θ) with a predominance of complexes containing combinations of αβγ or αβδ. The gene encoding the α<sub>1</sub> subunit (GABRA1) has been linked to an autosomal dominant form of juvenile myoclonic epilepsy. Mutations in GABRG2 encoding the γ<sub>2</sub> subunit have been associated with GEFS+ and the syndrome of childhood absence epilepsy with febrile seizures. Functional characterization of GABA<sub>A</sub> receptor subunit mutations in Xenopus oocytes and cultured mammalian cells demonstrated impaired receptor activity in vitro, suggesting reduced GABA<sub>A</sub>-mediated synaptic inhibition as a primary cause for neuronal hyperexcitability.

The third gene encoding a chloride-transporting protein associated with inherited epilepsy is CLCN2. The gene CLCN2 encodes a chloride channel that is widely distributed in the nervous system and has a suspected role in neuronal excitability. Mutations in CLCN2 have been associated with idiopathic generalized epilepsy in 3 families exhibiting autosomal dominant inheritance and multiple seizure phenotypes, including juvenile myoclonic epilepsy, juvenile absence epilepsy, childhood absence epilepsy, and epilepsy with grand mal seizures on awakening.

The CLCN2 gene was originally suspected based on genetic linkage data indicating that a region near chromosome 3q26 was a susceptibility locus for idiopathic generalized epilepsy, along with its suspected role in neuronal excitability. Two CLCN2 mutations completely abolish chloride channel function. However, a third mutation (G715E) enables chloride channel activation at less negative membrane potentials. Therefore, distinct cellular mechanisms may account for epilepsy associated with different CLCN2 mutations.

RELEVANCE TO NEUROSCIENCE AND THE PRACTICE OF NEUROLOGY

By defining the molecular basis of uncommon Mendelian epilepsy syndromes, we identify genes that may contribute to epileptogenesis in more common and genetically complex forms of the disease. Similarly, by studying the physiological impact of specific mutations, we discover the diversity of cellular mechanisms that can promote neuronal hyperexcitability and learn more about the importance of genes expressed in the brain. These advances also contribute to our ability to recognize and diagnose inherited neurological diseases, provide important information useful for counseling affected families, and identify potential new targets for anticonvulsant therapy.

Accepted for publication November 3, 2003.

This study was supported by Javits Neuroscience Investigator Award NS32387 from the National Institute of Neurological Disorders and Stroke, Bethesda, Md.

I thank Christopher Lossin, PhD, for critical reading of the manuscript.

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