Isolating Epilepsy Genes and Their Comorbidities

Jeffrey L. Noebels, MD, PhD

Departments of Neurology, Neuroscience, and Molecular and Human Genetics, Baylor College of Medicine, One Baylor Plaza, Houston, TX, 77030 USA

Key words: Epilepsy, Dementia, SUDEP

Published online April 7, 2010

Abstract

The number of genes underlying monogenic epilepsies in human and animal models continues to grow as the clinical syndromes for a variety of heritable epilepsies yield to neurogenetic analysis. Interestingly, the diversity of co-morbid phenotypes linked to single epilepsy gene defects is also expanding. While many rare familial idiopathic epilepsy disorders are typically defined as “pure” seizure syndromes, many occur in the context of more complex clinical symptomatology. This is not surprising, since genes that alter cortical excitability are expressed in diverse brain networks, as well as in other tissues. In this brief review, two such genes are described. The gene encoding amyloid precursor protein along with related genes contributing to the accumulation of an aberrant peptide cleavage product, Aβ42, has now been placed in a molecular pathway mediating both the dementia of Alzheimer’s Disease and seizures. A second gene, KCNQ1, encoding one of the voltage-sensitive ion channels contributing to membrane repolarisation in the heart, is co-expressed in both heart and brain where mutations contribute to cardiac arrhythmias, early mortality, and seizures. KCNQ1, a member of the cardiac LQTS gene family, is the first validated candidate gene for...
sudden unexplained death in epilepsy (SUDEP). The new strategy of searching for epilepsy genes linked to non-epileptic co-morbidity is providing illuminating examples of the role of neuronal network hyperexcitability in explaining the clinical spectrum of complex human neurological disorders that feature seizures.

Introduction

The definition of idiopathic epilepsy as a condition of isolated cortical seizures in the absence of any other neurological or medical disorder probably arose from the understandable eagerness of clinical epidemiologists to adopt a simplified conceptual framework to describe the human disease. In contrast, a more realistic perception of epilepsy held by clinicians who care for individuals with seizure disorders is: “I wish seizures were the only problem”. In fact, a wide variety of co-morbid symptoms accompany seizures in many individuals with epilepsy, including psychiatric, neurological, and medical concerns. These problems may precede, coincide with, or follow the onset of seizures themselves. The more common include depression, anxiety, migraine, cognitive dysfunction, and premature unexplained death, among others. In most instances it is difficult for the clinician, particularly without an informative family history, to determine whether these co-morbidities arise from a single defect, or are coincidental findings unrelated to the pathogenesis of the seizure disorder. The co-morbidity may also be a product of the seizures themselves, or even reflect idiosyncratic side-effects of anti-epileptic medication.

One possible approach to deciphering this complex problem is to search for single genes with dual phenotypes that can give rise to epilepsy linked to a second deficit. This reverse approach to identifying epilepsy genes by focusing on their ability to produce specific co-morbid states is but one additional way to enrich our understanding of the complex biology and clinical presentation of epilepsy as more than just a disorder defined by electroencephalographic discharges. The molecular diagnosis of co-morbidities also expands the clinical classification of syndromes and may alert the clinician to potential preventative therapy or otherwise alter their clinical management. Here I review two recent examples of translational research identifying candidate genes underlying ‘epilepsy spectrum disorders’ that illustrate this neurogenetic approach to clinical epileptology.

Epilepsy in Alzheimer’s Disease – the Role of APP

The presence of seizures in individuals with Alzheimer type dementia (AD) has been noted for many years and recently has become the subject of closer epidemiological scrutiny. An increased incidence of seizures is most often found in younger persons with early onset dementia (50–60 years), but even in the more elderly with advanced dementia the incidence of seizures is increased at least three-fold over age-matched populations (1). In the majority of AD patients, these seizures are classified as partial complex (2). Although well documented, the biological basis for sei-
zures in what has been traditionally held to be a progressive, purely neurodegenerative disorder has never been clearly explained, and the mechanisms for hyperexcitability are unknown. A commonly held view is that dementia and seizures represent fundamentally independent disorders, since overt seizures are not uniformly present in all AD patients, nor do all patients with epilepsy develop progressive cognitive deficits. They could represent the unfortunate co-occurrence of two disease genes, or the non-specific accumulation of a variety of unrelated secondary brain ischaemic micro-lesions, since the cardinal pathological features of advanced AD are the presence of cortical atrophy and cell death. Alternatively, seizures could arise from highly specific neuronal excitability changes related to the molecular pathogenesis of certain subtypes of dementia. Clinical dementias, like epilepsies, have multiple aetiologies, and at present it is not known whether the increased epidemiological incidence of epilepsy in Alzheimer’s-type dementia is biologically linked or a simple coincidence.

New Mechanistic Insight from Non-convulsive Seizures in AD Mouse Models

Important evidence regarding the relationship between a subset of dementia and epilepsy is finally emerging from the experimental analysis of transgenic AD mouse models. There are several transgenic mouse models of AD based upon overexpression of Aβ, a peptide cleavage product of the amyloid precursor protein APP, and all show varying degrees of hippocampal and cortical pathology, including plaque deposition and hyperphosphorylated tau protein, along with behavioral deficits in cognitive performance (3). While early neurophysiological studies of these models were focused on synaptic plasticity, recent analysis of network level activity now reveals that the gene encoding amyloid precursor protein (APP) can be considered to define a novel gene pathway for epilepsy, although the pathogenic steps are certainly more complex than for those, for example, that underlie inherited ion channel disorders. Rather than directly mediating intrinsic neuronal membrane excitability, the major biochemical steps elucidated in the pathogenesis of AD begin with the pathological accumulation of various forms of amyloid-β (Aβ) peptide at both extracellular and intracellular sites. In some individuals, this pathogenic accumulation is due to inherited mutations in APP genes as well as those mediating its proteolytic cleavage and clearance. Extracellular accumulation leads to increased Aβ peptide levels, amyloid plaques, intracellular neuritic dystrophic tangles, widespread synaptic and neuronal loss affecting many neurotransmitter pathways, and ultimately, through multiple but still unclear mechanisms, to the slow onset of clinical cognitive deterioration. Not all individuals with amyloid plaques are demented; in fact amyloid plaques are present in cognitively unimpaired as well as mildly impaired individuals (4). While the physiological function of the native amyloid precursor protein (APP) itself is still unclear, the Aβ peptide fragments can be released extracellularly upon intense synaptic activation (5), and are expressed in
virtually all brain regions throughout development. Thus, like several other neurodegenerative disorders, despite the selective vulnerability of certain brain regions for amyloid plaque accumulation, the expression pattern of the causative gene itself provides little indication of the regional cortical deficits that define the emergent cognitive disorder.

**Network Remodeling and Hyperexcitability in AD mouse models**

Based on a growing appreciation of network level dysfunction in AD, Palop et al. (6) examined a mouse model engineered to over-express a gene containing a human familial Alzheimers Disease (FAD) mutation, the J20 mouse, and discovered striking evidence for hippocampal network axon remodeling that is similar, but not identical to, the changes identified in both patients with temporal lobe epilepsy and experimental models of hippocampal seizures, thus offering new insight into potential mechanisms underlying dementia. The cellular changes included loss of the calcium binding protein calbindin in dentate granule cells, and ectopic sprouting of their mossy fibres – two structural alterations long known to be present in human and experimental models of temporal lobe epilepsy and believed to be the direct result of excess glutamate toxicity and seizure activity itself. An additional important feature noted in the AD models was the sprouting of fibers containing the inhibitory neurotransmitter NPY. Interestingly, these patterns of synaptic reorganization are also similar to those identified in human and experimental models of temporal lobe epilepsy (Figure 1) (7, 8).

In order to ascertain whether an AD mouse model with these patterns of aberrant axonal reorganization occurred in the presence of neuronal hypersynchronous discharge activity, chronic EEG monitoring was performed in awake J20 mice in the Noebels laboratory. These recordings revealed evidence of abnormal synchronous interictal EEG discharges, both cortical and hippocampal, as well as clinical seizure activity of two types. The most frequent seizures were non-convulsive without behavioral arrest (Figure 2); in addition, rare and unprovoked motor convulsions were also observed. EEG seizures have been confirmed in other AD mouse models (9). These transgenic mice therefore demonstrate that simple genetic overexpression of a human mutant form of Aβ is sufficient to create an epileptogenic lesion, and raise the question whether similar epileptic patterns may be present in human AD. The appearance of non-convulsive seizure semiology was a novel observation that also raised the question of whether abnormal hippocampal neuronal synchronization might go clinically undetected in human AD patients, and indeed whether this network level abnormality might contribute to the cognitive decline in at least a subset of patients with AD, since in epilepsy the deleterious effects of temporal lobe seizures on cognitive performance are well established.

In patients with Alzheimer’s disease, scalp-recorded EEG patterns typically show abnormalities predominantly composed of slowed background rhythms, and cortical interictal discharges are unusual in routine EEG
scalp recordings from individuals with idiopathic dementia. Even in patients with confirmed temporal lobe epilepsy, scalp-recorded interictal discharges are seen in fewer than half the cases (10). Three factors may lead to this apparent lack of evidence for cerebral hyperexcitability in AD patients in contrast to mouse AD models. First, EEG studies are not routinely performed in individuals with cognitive decline, and in any event would typically consist of a brief sample of activity rather than prolonged monitoring. Second, multiple pathogenic mechanisms contribute to human dementia, including vascular, nutritional, and other degenerative processes, and even within the approximately one half of this population that will be diagnosed with AD, there are multiple genetic subsets. Most AD

Figure 1. Network Remodeling in Alzheimer Disease and Temporal Lobe Epilepsy Models
Diagram comparing features of hippocampal remodeling present in transgenic mice overexpressing a human APP gene mutation with those present in a convulsant temporal lobe epilepsy model as induced by pilocarpine. Patterns of molecular plasticity, such as decreased calbindin in dentate granule cells, along with axonal reorganization of mossy fibers and NPY-expressing fibers are identified in both models, with subtle differences in some cases. Adapted from Palop, et al, 2007).
cases are sporadic, and true familial cases comprise a rare cause of AD. Thus few individuals with dementia who are examined by EEG have been genotypically proven to have mutations in the APP gene. Third, detection of seizure activity originating in human medial temporal lobe is difficult without intracerebral electrode recordings (10). Unfortunately, other non-invasive measures of human temporal lobe ictal activity such as magnetoencephalography are also relatively insensitive to aberrant electrical activity in the medial temporal lobe (11).

Dissecting the Aβ Lesion to Identify Therapeutic Targets

At the cellular and molecular levels, the Aβ-induced lesion in neural networks is complex, and a detailed accounting of the necessary and sufficient pathological changes for the development of both seizures and dementia remain to be determined. One obvious

Figure 2. Non-convulsive seizure in Aβ overexpressing mouse model
EEG recording of spontaneous non-convulsive seizure in adult transgenic mouse overexpressing a variant form of the amyloid precursor protein (hAPPJ20) bearing a human mutation leading to familial Alzheimer’s Disease. Spontaneous seizure activity is first seen in depth electrodes implanted bilaterally in the hippocampal formation (LH/RH). From Palop et al., (2007).
question is whether the structural plasticity found in the hAPP/J20 hippocampus is the cause or a result of aberrant hyperexcitability. While the appropriate serial pathological studies remain to be done, significant differences between the patterns observed in J20 mice and those in purely convulsant experimental models of epilepsy such as pilocarpine- or kainate-induced seizures suggest that hippocampal network remodelling may vary considerably according to the convulsant model and the stage when it is examined. For example, J20 mice show little or no cell loss, and the sprouting of mossy fibres do not fully extend into the inner molecular layer of the dentate gyrus as commonly seen in convulsant models of epilepsy. Instead, the sprouted mossy fiber axons show preferential termination on inhibitory basket cell interneurons. These basket cells project to granule cells, and there is evidence for increased inhibition of the granule cell population. In addition, not only are hilar interneurons spared in the hippocampus, but those positive for NPY peptide show a pattern of axon terminal sprouting not described in convulsant models.

The transgenic AD model shows that APP/Aβ accumulation induces aberrant excitatory neuronal network activity in vivo and triggers complex molecular patterns of compensatory inhibitory and excitatory mechanisms in hippocampal circuitry. Both the aberrant excitatory neuronal activity and the compensatory inhibitory signalling contribute to AD-related network dysfunction, and, at least in J20 mice, lead to clear evidence of network hypersynchrony, including interictal discharges and generalized non-convulsive seizures. It is not entirely clear which process, the network remodelling or the network hyperexcitability initiates the pathological cascade. Mutant APP/Aβ-induced downstream network remodelling is likely to occur slowly over the course of the disease, and could contribute at some critical stage to episodic instability and dysrhythmia in hippocampal circuits. Alternatively, the electrical instability could be present at early stages, and drive the remodelling process, as it does in other convulsant-induced seizure models.

Since it is entirely possible that each of these two maladaptive reactive processes begets the other, it may be difficult to assign a clearly active or passive role to either. Nevertheless, since this pathogenic “vicious cycle” may amplify dysfunction within the neural circuit, it is also intuitively clear that interrupting either one could prove to be an important, if not critical, step in slowing the progression of cognitive decline in individuals with Aβ pathology. Further translational exploration of these issues in animals and human studies over the coming decade will determine which patients may benefit from new therapeutic approaches directed at suppressing aberrant network excitability in the temporal lobe.

Epilepsy and Cardiac Arrhythmias: The Role of KCNQ1 in SUDEP

Our laboratory has recently described a second example illustrating how the study of a compound clinical epilepsy syndrome can lead to the identification of a novel gene for
epilepsy. In this case, the gene links a shared excitability phenotype in the heart and brain, thus identifying a candidate monogenic cause of a lethal co-morbidity in idiopathic epilepsy, namely, sudden unexplained death (12). Sudden unexplained death in epilepsy (SUDEP) is one of the most common causes of mortality in idiopathic seizure disorders, accounting for 2-18% of deaths in various studies. Despite multiple epidemiological risk factors (including early onset intractable seizures, polypharmacy, and an age range of 20-40 years of age), non-specific autopsy findings, and a long series of case reports suggesting the possibility of autonomic dysregulation and cardiac arrhythmias, no convincing molecular link between seizures and SUDEP has yet been identified (13).

Cardiac Long QT interval Genes, Ion Channels and Seizures

Like epilepsies, clinical syndromes defined by syncope in both children and young adults accompanied by characteristic electrocardiographic signatures have led to the identification of a variety of genes underlying cardiac syncope, and sudden unexpected death. A major category includes the long QT interval syndrome (LQTS). Genes for 12 mendelian syndromes underlying this episodic disorder have been identified, of which five encode voltage-gated ion channels responsible for cardiac repolarization (14). The incidence of LQT has been estimated at 1 in 2500 individuals (15).

We evaluated the unifying hypothesis that mutation of a single gene responsible for excitability and synchronization, if co-expressed in heart and brain, could provoke cardiac and neuronal repolarization defects leading to a combined syndrome of epilepsy and cardiac arrhythmias. Such individuals might be at increased risk of lethal cardiac compromise and SUDEP. In order to test this hypothesis, we first examined the ion channel genes linked to cardiac long QT interval syndromes (LQTS), since these genes have already been demonstrated to lead to malignant arrhythmias and sudden death. We determined that all of these genes except two, $\text{KCNQ1}$ and $\text{KCNE1}$, had been shown to be present not only in cardiac tissue but also in the central nervous system. $\text{KCNQ1}$ encodes a voltage-gated potassium channel KvLQT1 that is the most common gene linked to human LQTS. $\text{KCNE1}$ (MinK) is a potassium channel subunit that interacts with $\text{KCNQ1}$. Interestingly, $\text{KCNQ1}$ is a member of the family of Kv7 potassium channels that includes two other members, $\text{KCNQ2}$ and $\text{KCNQ3}$, both of which have been linked to monogenic forms of neonatal epilepsy (15). Using PCR, specific antibodies, and co-immunoprecipitation studies, our laboratory demonstrated that both $\text{KCNQ1}$ as well as the $\text{KCNE1}$ subunit were present in mouse and human brain, suggesting a potential role of these two interacting LQT genes in epileptogenesis. We next determined that two independent transgenic mouse lines, each expressing a human $\text{KCNQ1}$ mutation that had been linked to the human LQT1 syndrome showed spontaneous seizures during prolonged neurophysiologic monitoring of awake and behaving mice (Figure 3).
Figure 3. KCNQ1 and seizures in brain
(Left) Immunocytochemistry with specific Kcnq1 antibody reveals expression of Kcnq1 protein in mouse forebrain. (Right) EEG recordings of spontaneous electrographic seizure discharge in mouse mutant knockin model bearing human LQT1 mutation (lower), compared with absence of these seizures in wild type littermate mouse (upper). From Goldman et al, 2009.

Figure 4. Brain/Heart Interactions in Kcnq1 mouse mutant
(Left) Specific antibody staining of mouse brain stem reveals presence of Kcnq1 channel protein in vagal nerve nuclei. (Right) Simultaneous in vivo recordings of EEG (upper) and EKG (lower)activity in freely moving Kcnq1 mutant mouse display cardiac asystole coincident with EEG interictal discharge. This pattern was identified frequently in mutant mice, suggesting the possibility of vagal nerve hyperexcitability due to impaired repolarization. From Goldman et al, 2009.
Interestingly, the seizure phenotypes were complex; heterozygous \textit{Kcnq1} mutant mice displayed both non-convulsive seizures as well as both subtle partial seizures and generalized convulsive activity. We then used simultaneous EKG monitoring of awake and behaving mice to ascertain whether the expression of the human LQT1 mutations were arrhythmogenic, and to evaluate how these patterns might depend upon aberrant cortical discharges. We found that both \textit{Kcnq1} mutant mouse lines displayed frequent cardiac arrhythmias during normal exploratory behavior. The patterns of interictal and ictal cardiac arrhythmias were notable for benign sinus tachy- and brady arrhythmias, as well as more malignant variants including asystoles. Interestingly, although both brain neural networks as well as cardiac pacemaking tissue displayed temporally-independent excitability defects, abnormal discharges were frequently coupled between heart and brain (Figure 4). The localization of the KvLQT1 channel in vagal nerve nuclei in the brainstem provides one possible mechanism for this tight coupling between cortical discharges and autonomic outflow, since defective repolarization in these axons could lead to repetitive firing and excessive parasympathetic transmission.

Finally, during prolonged monitoring sessions, one episode of SUDEP was captured in a \textit{Kcnq1} mutant, consisting of a prolonged seizure, followed by EEG flattening, and finally severe bradycardia and asystole (12). This general pattern resembled the solitary description of a human SUDEP event reported in the literature with simultaneous EEG/EKG recordings (16).

**New Candidate Genes for SUDEP**

The discovery of the molecular link between the co-morbidity of arrhythmias in heart and brain in this mouse model of human LQT syndrome opens a number of strategic avenues for future research and therapeutic opportunities for clinical translation. First, while the clinical descriptions of often unwitnessed syncopy versus seizures in LQT patients have long presented a challenge to the diagnostician, it is now evident that the same inherited disorder of membrane excitability that alters cardiac pacemaking can directly provoke an epilepsy phenotype. The important lesson for the cardiologist is therefore that the presence of verified clinical syncopy when demonstrated by testing in a symptomatic individual with LQT does not preclude the possibility that other prior or future episodes of dizziness or falls may in fact represent true cerebral seizures. LQT1 is the most common gene for human LQT, and clinical assessment of patients with genotyped mutations in this and other LQT genes has shown a clearly increased incidence of “seizure-like episodes” along with cardiac syncopy (17). Similarly, for the epileptologist, the finding of abnormal EEG and clinical seizures should prompt a detailed search for family history of sudden death, syncopy or other cardiac disorder, and a routine cardiology evaluation including 12 lead EKG, followed by genetic testing where indicated.

Second, since the remaining LQT ion
channels linked to this phenotype have all also been demonstrated in human brain, *KCNQ1* represents a molecular “proband” in this gene family, thus expanding the candidate gene list for SUDEP. Validating these remaining genes will be important to improve risk prediction and counseling for individuals with idiopathic epilepsy. In the laboratory, the availability of a validated animal model of human LQT1 SUDEP, as well as others, will permit EKG screening of the relative toxicities of current antiepileptic therapy, as well as the utility of protective antiarrhythmic pharmacology.

**Conclusions**

New mouse models of epilepsy syndromes that include clinical co-morbidity – one with cognitive deficits caused by an accumulation of Aβ peptide, the other with cardiac arrhythmias and sudden unexpected death – serve as excellent illustrations of the complex excitability changes that can be triggered by a single gene lesion. In both models, interestingly, the epileptic disorder features not only clinically recognizable convulsive seizures, but also non-convulsive patterns, a clinically silent phenotype that is very likely to go unrecognized for prolonged periods of time. Although it is often difficult to extrapolate clinical phenotypes from mouse to human, the appearance of this seizure type in two very different experimental models suggests that co-morbid epilepsy syndromes may be underestimated in both man and mouse. The models themselves provide an important opportunity to trace the molecular pathogenesis of epilepsy associated with these novel genes and suggest future gene-directed improvements in the treatment of these syndromes.

**Acknowledgements**

This research is supported by the National Institutes of Health and the Blue Bird Circle Foundation for Pediatric Neurology. I thank Drs. Alica Goldman, Ed Glasscock, Tim Chen and Tara Klassen for their contribution to this research.

**References**


51


