The LGI1/epitempin gene encodes two protein isoforms differentially expressed in human brain.


CNR-Istituto di Neuroscienze, Sezione di Padova, Dipartimento di Scienze Biomediche Sperimentali, Università di Padova, Padua, Italy.

The leucine-rich, glioma inactivated 1 (LGI1)/Epitempin gene has been linked to two phenotypes as different as gliomagenesis and autosomal dominant lateral temporal epilepsy. Its function and the biochemical features of the encoded protein are unknown. We characterized the LGI1/Epitempin protein product by western blot analysis of mouse and human brain tissues. Two proteins of about 60 and 65 kDa were detected by an anti-LGI1 antibody within the expected molecular mass range. The two proteins appeared to reside in different subcellular compartments, as they were fractionated by differential centrifugation. The specificity of both polypeptides was validated by cell transfection assay and mass spectrometry analysis. Immunoblot analysis of protein distribution in various zones of the human brain revealed variable amounts of both proteins. Notably, these proteins were more abundant in the temporal neocortex than in the hippocampus, the difference in abundance of the 65-kDa product being particularly pronounced. These results suggest that the two protein isoforms encoded by LGI1/Epitempin are differentially expressed in the human brain, and that higher expression levels of these proteins in the lateral temporal cortex may underlie the susceptibility of this brain region to the epileptogenic effects of LGI1/Epitempin mutations.

PMID: 16787412 [PubMed - indexed for MEDLINE]
Increased sensitivity of the neuronal nicotinic receptor alpha 2 subunit causes familial epilepsy with nocturnal wandering and ictal fear.


Human Molecular Genetics Unit, Dibit San Raffaele Scientific Institute, Milan, Italy.

Sleep has traditionally been recognized as a precipitating factor for some forms of epilepsy, although differential diagnosis between some seizure types and parasomnias may be difficult. Autosomal dominant frontal lobe epilepsy is characterized by nocturnal seizures with hyperkinetic automatisms and poorly organized stereotyped movements and has been associated with mutations of the alpha 4 and beta 2 subunits of the neuronal nicotinic acetylcholine receptor. We performed a clinical and molecular genetic study of a large pedigree segregating sleep-related epilepsy in which seizures are associated with fear sensation, tongue movements, and nocturnal wandering, closely resembling nightmares and sleep walking. We identified a new genetic locus for familial sleep-related focal epilepsy on chromosome 8p12.3-8q12.3. By sequencing the positional candidate neuronal cholinergic receptor alpha 2 subunit gene (CHRNA2), we detected a heterozygous missense mutation, I279N, in the first transmembrane domain that is crucial for receptor function. Whole-cell recordings of transiently transfected HEK293 cells expressing either the mutant or the wild-type receptor showed that the new CHRNA2 mutation markedly increases the receptor sensitivity to acetylcholine, therefore indicating that the nicotinic alpha 2 subunit alteration is the underlying cause. CHRNA2 is the third neuronal cholinergic receptor gene to be associated with familial sleep-related epilepsies. Compared with the CHRNA4 and CHRNB2 mutations reported elsewhere, CHRNA2 mutations cause a more complex and finalized ictal behavior.

PMID: 16826524 [PubMed - indexed for MEDLINE]

PMCID: PMC1559502
Genetic analysis of the LGI/Epitempin gene family in sporadic and familial lateral temporal lobe epilepsy.


Unitat de Genètica Molecular, Dept. de Genòmica i Proteòmica, Institut de Biomedicina de València - CSIC, Jaume Roig, 11. E46010 València, Spain.

Mutations in the LGI1/Epitempin gene cause autosomal dominant lateral temporal lobe epilepsy (ADLTE), a partial epilepsy characterized by the presence of auditory seizures. However, not all the pedigrees with a phenotype consistent with ADLTE show mutations in LGI1/Epitempin, or evidence for linkage to the 10q24 locus. Other authors as well as ourselves have found an internal repeat (EPTP, pfam# PF03736) that allowed the identification of three other genes sharing a sequence and structural similarity with LGI1/Epitempin. In this work, we present the sequencing of these genes in a set of ADLTE families without mutations in both LGI1/Epitempin and sporadic cases. No analyzed polymorphisms modified susceptibility in either the familial or sporadic forms of this partial epilepsy.

PMID: 16707245 [PubMed - indexed for MEDLINE]
Linkage analysis and disease models in benign familial infantile seizures: a study of 16 families.


Laboratory of Neurogenetics, Unit of Muscular and Neurodegenerative Disease, Istituto G. Gaslini, University of Genova, Genova, and Division of Neurology, Ospedale Pediatrico Bambino Gesù, Roma, Italy.

PURPOSE: Benign familial infantile seizures (BFIS) is a genetically heterogeneous condition characterized by partial seizures, onset age from 3 to 9 months, and favorable outcome. BFIS loci were identified on chromosomes 19q12-13.1 and 16p12-q12, allelic to infantile convulsions and choreathetosis. The identification of SCN2A mutations in families with only infantile seizures indicated that BFIS and BFIS may show overlapping clinical features. Infantile seizures also were in a family with familial hemiplegic migraine and mutations in the ATP1A2 gene. We have examined the heterogeneous genetics of BFIS by means of linkage analysis. METHODS: Sixteen families were examined. Probands underwent neurologic examination, at least one EEG recording, and, when possible, brain CT and MRI. Clinical information about relatives was collected. Families with SCN2A or ATP1A2 mutations were excluded from the study. Chromosome 16p and 19q loci were examined by linkage analysis using two models that differed in penetrance rate. Genetic heterogeneity was evaluated with both models. RESULTS: Clinical information was available for 124 members of affected families. BFIS was diagnosed in 69 subjects. One patient without BFIS had a single febrile seizure, and another had rare episodes of paroxysmal dystonia. Evidence of linkage was obtained only for chromosome 16. Moreover, the high penetrance allowed the identification of genetic heterogeneity. CONCLUSIONS: Our data confirm the relevance of the chromosome 16 locus in BFIS and suggest the presence of an additional locus. This study shows that the genetic model used affects the outcome of linkage analysis.

PMID: 16822249 [PubMed - indexed for MEDLINE]
Effects in neocortical neurons of mutations of the Na(v)1.2 Na+ channel causing benign familial neonatal-infantile seizures.


Department of Neurophysiopathology, Istituto Neurologico C. Besta, 20133 Milan, Italy.

Mutations of voltage-gated Na+ channels are the most common cause of familial epilepsy. Benign familial neonatal-infantile seizures (BFNIS) is an epileptic trait of the early infancy, and it is the only well characterized epileptic syndrome caused exclusively by mutations of Na(V)1.2 Na+ channels, but no functional studies of BFNIS mutations have been done. The comparative study of the functional effects and the elucidation of the pathogenic mechanisms of epileptogenic mutations is essential for designing targeted and effective therapies. However, the functional properties of Na+ channels and the effects of their mutations are very sensitive to the cell background and thus to the expression system used. We investigated the functional effects of four of the six BFNIS mutations identified (L1330F, L1563V, R223Q, and R1319Q) using as expression system transfected pyramidal and bipolar neocortical neurons in short primary cultures, which have small endogenous Na+ current and thus permit the selective study of transfected channels. The mutation L1330F caused a positive shift of the inactivation curve, and the mutation L1563V caused a negative shift of the activation curve, effects that are consistent with neuronal hyperexcitability. The mutations R223Q and R1319Q mainly caused positive shifts of both activation and inactivation curves, effects that cannot be directly associated with a specific modification of excitability. Using physiological stimuli in voltage-clamp experiments, we showed that these mutations increase both subthreshold and action Na+ currents, consistently with hyperexcitability. Thus, the pathogenic mechanism of BFNIS mutations is neuronal hyperexcitability caused by increased Na+ current.

PMID: 17021166 [PubMed - indexed for MEDLINE]
Brain MRI findings in severe myoclonic epilepsy in infancy and genotype-phenotype correlations.

INTRODUCTION: To determine the occurrence of neuroradiological abnormalities and to perform genotype-phenotype correlations in severe myoclonic epilepsy of infancy (SMEI, Dravet syndrome).

PATIENTS AND METHODS: Alpha-subunit type A of voltage-gated sodium channel (SCN1A) mutational screening was performed by denaturing high-performance liquid chromatography (DHPLC) and multiplex ligation probe amplification (MLPA). MRI inclusion criteria were: last examination obtained after the age of 4 years on 1.5-T systems; hippocampal cuts acquired perpendicular to the long axis of the hippocampus; qualitative assessment was performed on T(1)-weighted, T(2)-weighted, proton density, and 1-3 mm thick coronal FLAIR images.

RESULTS: We collected 58 SMEI patients in whom last MRI was performed at or later than 4 years of age. SCN1A mutations occurred in 35 (60%) cases. Thirteen (22.4%) out of 58 patients showed abnormal MRIs. Eight patients showed cortical brain atrophy of which 3 associated to ventricles abnormalities, 1 to cerebellar atrophy, 1 to white matter hyperintensity; 3 patients had ventricles enlargement only; 1 patient showed hippocampal sclerosis (HS); 1 had focal cortical dysplasia. Genotype-phenotype analysis indicated that abnormal MRIs occurred more frequently in patients without SCN1A mutations (9/23; 39.1%) compared to those carrying SCN1A mutations (4/35; 11.4%) (p=0.02).

CONCLUSION: Different brain abnormalities may occur in SMEI. Only one case with HS was observed; thus, our study does not support the association between prolonged febrile seizures and HS in SMEI. Abnormal MRIs were significantly more frequent in patients without SCN1A mutations. Prospective MRI studies will assess the etiological role of the changes observed in these patients.

PMID: 17381446 [PubMed - indexed for MEDLINE]
A de novo LGI1 mutation causing idiopathic partial epilepsy with telephone-induced seizures.


Department of Neurosciences, Bellaria Hospital, Bologna, Italy.

PMID: 17562837 [PubMed - indexed for MEDLINE]
Mutational analysis of EFHC1 gene in Italian families with juvenile myoclonic epilepsy.


Institute of Neurological Sciences, National Research Council, Mangone-Cosenza, Italy.

OBJECTIVES: Mutations in the EFHC1 gene have been reported in six juvenile myoclonic epilepsy (JME) families from Mexico and Belize. In this study, we screened 27 unrelated JME Italian families for mutations in the EFHC1 gene. MATERIALS AND METHODS: Twenty-seven families (86 affected individuals, 52 women) with at least two affected members with JME were selected. DNA was isolated from peripheral blood lymphocytes by standard methods and each exon of the EFHC1 gene was amplified and sequenced using intronic primers. RESULTS: Two heterozygous mutations were identified in three unrelated families. One (R353 W) was a novel missense mutation, while the F229 L mutation was previously described (say which one of the two occurred in two families). Both mutations cosegregated with the disease. In a fourth family, the variant 545G-->A (resulting in the amino acid substitution R182 H) cosegregated with JME. CONCLUSIONS: The results of our study extend the distribution of EFHC1 mutations to the white population and confirm the high level of genetic heterogeneity associated with JME.

PMID: 17634063 [PubMed - indexed for MEDLINE]
Modulatory proteins can rescue a trafficking defective epileptogenic Nav1.1 Na+ channel mutant.


Department of Neurophysiopathology, Besta Neurological Institute, 20133 Milan, Italy.

Familial epilepsies are often caused by mutations of voltage-gated Na+ channels, but correlation genotype-phenotype is not yet clear. In particular, the cause of phenotypic variability observed in some epileptic families is unclear. We studied Na(v)1.1 (SCN1A) Na+ channel alpha subunit M1841T mutation, identified in a family characterized by a particularly large phenotypic spectrum. The mutant is a loss of function because when expressed alone, the current was no greater than background. Function was restored by incubation at temperature <30 degrees C, showing that the mutant is trafficking defective, thus far the first case among neuronal Na+ channels. Importantly, also molecular interactions with modulatory proteins or drugs were able to rescue the mutant. Protein-protein interactions may modulate the effect of the mutation in vivo and thus phenotype; variability in their strength may be one of the causes of phenotypic variability in familial epilepsy. Interacting drugs may be used to rescue the mutant in vivo.

PMID: 17928445 [PubMed - indexed for MEDLINE]
Different spectra of genomic deletions within the CCM genes between Italian and American CCM patient cohorts.

Liquori CL, Penco S, Gault J, Leedom TP, Tassi L, Esposito T, Awad IA, Frati L, Johnson EW, Squitieri F, Marchuk DA, Gianfrancesco F.

Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, NC, USA.

Cerebral cavernous malformations (CCMs) are vascular abnormalities of the brain that can result in hemorrhagic stroke and seizures. Familial forms of CCM are inherited in an autosomal-dominant fashion, and three CCM genes have been identified. We recently determined that large genomic deletions in the CCM2 gene represent 22% of mutations in a large CCM cohort from the USA. In particular, a 77.6 kb deletion spanning CCM2 exons 2-10 displays an identical recombination event in eight CCM probands/families and appears to be common in the US population. In the current study, we report the identification of six additional probands/families from the USA with this same large deletion. Haplotype analysis strongly suggests that this common deletion derives from an ancestral founder. We also examined an Italian CCM cohort consisting of 24 probands/families who tested negative for mutations in the CCM1, CCM2, and CCM3 genes by DNA sequence analysis. Surprisingly, the common CCM2 deletion spanning exons 2-10 is not present in this population. Further analysis of the Italian cohort by multiplex ligation-dependent probe analysis identified a total of ten deletions and one duplication. The overall spectrum of genomic rearrangements in the Italian cohort is thus quite different than that seen in a US cohort. These results suggest that there are elements within all three of the CCM genes that predispose them to large deletion/duplication events but that the common deletion spanning CCM2 exons 2-10 appears to be specific to the US population due to a founder effect.

PMID: 18060436 [PubMed - in process]
Analysis of LGI1 promoter sequence, PDYN and GABBR1 polymorphisms in sporadic and familial lateral temporal lobe epilepsy.


CNR-Institute of Neurosciences, Section of Padua, Padova, Italy.

Autosomal dominant lateral temporal epilepsy (ADTLE) is a genetically transmitted epileptic syndrome characterized by focal seizures with predominant auditory symptoms likely originating from the lateral region of the temporal lobe. Mutations in coding region or exon splice sites of the leucine-rich, glioma-inactivated 1 (LGI1) gene account for about 50% of ADLTE families. De novo LGI1 mutations of the same kind have also been found in about 2.5% of non-familial cases with idiopathic partial epilepsy with auditory features (IPEAF). In both conditions, mutations in the LGI1 promoter region have not been reported. We sequenced the minimal promoter region of LGI1 in the probands of 16 ADLTE families and in 104 sporadic IPEAF patients and no mutations clearly linked to the disease were found. However, two polymorphisms, -500G>A and -507G>A, with potential functional implications were identified and analysed in the cohort of sporadic IPEAF patients but their frequencies did not differ from those found in a control population of similar age, gender and geographic origin. We also analysed in our study population the GABA(B) receptor 1 c.1465G>A and the prodynorphin promoter 68-bp repeat polymorphisms, previously associated with temporal lobe epilepsy. None of these polymorphisms showed a significant association with IPEAF, whereas a tendency towards association with the prodynorphin low expression (L) alleles was found in the small group of ADLTE index cases, in agreement with previous studies suggesting that this polymorphism is a susceptibility factor in familial forms of temporal lobe epilepsy.

PMID: 18355961 [PubMed - in process]
Familial mesial temporal lobe epilepsy (FMTLE) : a clinical and genetic study of 15 Italian families.


Epilepsy Center, Department of Neurological Sciences Federico II University, Via Pansini 5, 80131, Napoli, Italy.

**INTRODUCTION :** Familial mesial temporal lobe epilepsy (FMTLE) is characterized by prominent psychic and autonomic seizures, often without hippocampal sclerosis (HS) or a previous history of febrile seizures (FS), and good prognosis. The genetics of this condition is largely unknown. We present the electroclinical and genetic findings of 15 MTLE Italian families.

**METHODS :** FMTLE was defined when two or more first-degree relatives had epilepsy suggesting a mesial temporal lobe origin. The occurrence of seizures with auditory auras was considered an exclusion criterion. Patients underwent video-EEG recordings, 1.5-Tesla MRI particularly focused on hippocampal analysis, and neuropsychological evaluation. Genetic study included genotyping and linkage analysis of candidate loci at 4q, 18q, 1q, and 12q as well as screening for LGI1/Epitempin mutations.

**RESULTS :** Most of the families showed an autosomal dominant inheritance pattern with incomplete penetrance. Fifty-four (32 F) affected individuals were investigated. Twenty-one (38.8 %) individuals experienced early FS. Forty-eight individuals fulfilled the criteria for MTLE. Epigastric/visceral sensation (72.9 %) was the most common type of aura, followed by psychic symptoms (35.4 %), and déjà vu (31.2 %). HS occurred in 13.8% of individuals, three of whom belonged to the same family. Prognosis of epilepsy was generally good. Genetic study failed to show LGI1/Epitempin mutations or significative linkage to the investigated loci.

**DISCUSSION :** FMTLE may be a more common than expected condition, clinically and genetically heterogeneous. Some of the reported families, grouped on the basis of a specific aura, may represent an interesting subgroup on whom to focus future linkage studies.

PMID: 18004642 [PubMed - in process]
A novel loss-of-function LGI1 mutation linked to autosomal dominant lateral temporal epilepsy.


Muscular and Neurodegenerative Diseases Unit, Institute G. Gaslini, University of Genoa, Genoa, Italy.

BACKGROUND: Mutations responsible for autosomal dominant lateral temporal epilepsy have been found in the leucine-rich, glioma-inactivated 1 (LGI1) gene. OBJECTIVES: To describe the clinical and genetic findings in a family with autosomal dominant lateral temporal epilepsy and to determine the functional effects of a novel LGI1 mutation in culture cells. DESIGN: Clinical, genetic, and functional investigations. SETTING: University hospital and laboratory. PATIENTS: An Italian family with autosomal dominant lateral temporal epilepsy. MAIN OUTCOME MEASURE: Mutation analysis. RESULTS: A novel LGI1 mutation, c.365T>A (Ile122Lys), segregating with the disease was identified. The mutant Lgi1 protein was not secreted by culture cells. CONCLUSION: Our data provide further evidence that mutations in LGI1 hamper secretion of the Lgi1 protein, thereby precluding its normal function.

Familial epilepsy and developmental dysphasia: description of an Italian pedigree with autosomal dominant inheritance and screening of candidate loci.


Department of Neurosciences, Division of Neurology, Via Altura 3, Bellaria Hospital, 40139 Bologna, Italy. roberto.michelucci@ausl.bo.it

PURPOSE: To describe a familial epileptic condition combining a peculiar electro-clinical pattern with developmental language dysfunction in a large Italian kindred. METHODS: We studied the clinical and neurophysiological features of a 4-generation family with 10 affected members (3 deceased). We also analysed in 7 affected and 7 healthy members microsatellite markers for 51 candidate loci for epilepsy, including 42 loci containing ion channel genes expressed in the brain, as well as the SPCH1 and SRPX2 loci. RESULTS: Five of the seven living affected members (aged 20-58 years) had the full phenotype (seizures, EEG epileptiform abnormalities and dysphasia). The language dysfunction was the first symptom, becoming evident since the period of language development and mainly consisting of phonemic and syntactic paraphasias, difficulty of expression and reduced verbal fluency. The seizures had their onset between 2 and 23 years and were reported as epileptic falls (4) associated or not with myoclonic features, absences (3), tonic-clonic (1) and complex partial seizures (1). The seizures were easily controlled by antiepileptic treatment in all patients except one. In the five patients with a good response of seizures to treatment, the EEG...
tracings showed the coexistence of focal and generalized epileptiform abnormalities; in the refractory patient the interictal EEG demonstrated bilateral asynchronous frontotemporal paroxysms with left predominance and ictal SEEG recording suggested a multifocal origin of the discharges. MRI of the brain was normal in all patients. Linkage analysis provided negative LOD scores for all the investigated loci. CONCLUSION: We have described a novel familial pattern of epilepsy and developmental dysphasia which is not genetically linked to epilepsy or speech disorder loci, as documented by a candidate-gene linkage approach.

Autosomal dominant lateral temporal epilepsy: absence of mutations in ADAM22 and Kv1 channel genes encoding LGI1-associated proteins.


CNR-Institute of Neurosciences, Section of Padua, Padova, Italy.

Mutations in the LGI1 gene are linked to autosomal dominant lateral temporal epilepsy (ADLTE) in about half of the families tested, suggesting that ADLTE is genetically heterogeneous. Recently, the Lgi1 protein has been found associated with different protein complexes and two distinct molecular mechanisms possibly underlying ADLTE have been hypothesized: the one recognizes Lgi1 as a novel subunit of the presynaptic Kv1 potassium channel implicated in the regulation of channel inactivation, the other suggests that Lgi1 acts as a ligand that selectively binds to the postsynaptic receptor ADAM22, thereby regulating the glutamate-AMPA neurotransmission. Both mechanisms imply that LGI1 mutations result in alteration of synaptic currents, though of different types. Since their protein products have been found associated with Lgi1, the Kv1 channel subunit genes KCNA1, KCNA4, and KCNAB1 and ADAM22 can be considered strong candidates for ADLTE. We sequenced their coding exons and flanking splice sites in the probands of 9 carefully ascertained ADLTE families negative for LGI1 mutations. We failed to detect any mutation segregating with the disease, but identified several previously unreported polymorphisms. An association study of four non-synonymous variants (three found in ADAM22, one in KCNA4) in a population of 104 non-familial lateral temporal epilepsy cases did not show any modification of susceptibility to this disorder. Altogether, our results suggest that neither ADAM22 nor any of the three Kv1 channel genes are major causative genes for ADLTE.

Self-limited hyperexcitability: functional effect of a familial hemiplegic migraine mutation of the Nav1.1 (SCN1A) Na+ channel.

Cestèle S, Scalmani P, Rusconi R, Terragni B, Franceschetti S, Mantegazza M.
Familial hemiplegic migraine (FHM) is an autosomal dominant inherited subtype of severe migraine with aura. Mutations causing FHM (type 3) have been identified in SCN1A, the gene encoding neuronal voltage-gated Na(v)1.1 Na(+) channel alpha subunit, but functional studies have been done using the cardiac Na(v)1.5 isoform, and the observed effects were similar to those of some epileptogenic mutations. We studied the FHM mutation Q1489K by transfecting tsA-201 cells and cultured neurons with human Na(v)1.1. We show that the mutation has effects on the gating properties of the channel that can be consistent with both hyperexcitability and hypoexcitability. Simulation of neuronal firing and long depolarizing pulses mimicking promigraine conditions revealed that the effect of the mutation is a gain of function consistent with increased neuronal firing. However, during high-frequency discharges and long depolarizations, the effect became a loss of function. Recordings of firing of transfected neurons showed higher firing frequency at the beginning of long discharges. This self-limited capacity to induce neuronal hyperexcitability may be a specific characteristic of migraine mutations, able to both trigger the cascade of events that leads to migraine and counteract the development of extreme hyperexcitability typical of epileptic seizures. Thus, we found a possible difference in the functional effects of FHM and familial epilepsy mutations of Na(v)1.1.