Mutations in the \textit{LGI1}/\textit{Epitempin} gene on 10q24 cause autosomal dominant lateral temporal epilepsy

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Autosomal dominant lateral temporal epilepsy (EPT; OMIM 600512) is a form of epilepsy characterized by partial seizures, usually preceded by auditory signs. The gene for this disorder has been mapped by linkage studies to chromosomal region 10q24. Here we show that mutations in the \textit{LGI1} gene segregate with EPT in two families affected by this disorder. Both mutations introduce premature stop codons and thus prevent the production of the full-length protein from the affected allele. By immunohistochemical studies, we demonstrate that the \textit{LGI1} protein, which contains several leucine-rich repeats, is expressed ubiquitously in the neuronal cell compartment of the brain. Moreover, we provide evidence for genetic heterogeneity within this disorder, since several other families with a phenotype consistent with this type of epilepsy lack mutations in the \textit{LGI1} gene.

INTRODUCTION

Autosomal dominant lateral temporal epilepsy (EPT) refers to an epileptic syndrome characterized by focal seizures located in the lateral area of the temporal lobe, and is also termed lateral temporal epilepsy with acoustic aura or autosomal dominant partial epilepsy with auditory features. Ottman and colleagues \textsuperscript{1} provided the first description of this disorder. Clinically, this epileptic syndrome is characterized, in part, by auditory hallucinations, although the extent of patients experiencing these varies amongst the reported pedigrees (1,2). Other sensory symptoms are also present, such as visual hallucinations. The onset of the disease is quite variable even within pedigrees, although it usually affects young individuals. Seizures are
scarce, usually occur during sleep periods and are well controlled with standard anti-epileptic treatments.

In 1995, a kindred with this type of focal epilepsy was linked to a 10 cM interval on 10q23–q24 (1), this linkage was later confirmed, in 1999, by our group in a second large pedigree (2), further refining the linked area to a 3 cM interval between markers D10S185 and D10S577. In this paper, we report the identification of mutations in a gene, LG11, that segregate with the disease in two different pedigrees affected by EPT. In light of the proposed relationship between this gene and EPT, we propose a new, more descriptive, name for this gene: LG11/Epitempin (approved by the HUGO Nomenclature Committee; symbol LG11).

RESULTS

After further defining the region linked with EPT to a 3 cM interval, our group undertook both a positional cloning strategy and a candidate gene approach to identify the gene responsible of this form of focal epilepsy (3,4). Our candidate gene strategy took into account, in addition to the known or inferred function of a gene, the expression pattern of the genes mapped to this region. Thus we were interested in genes expressed (exclusively or not) in the brain and, within the central nervous system, at least in the lateral part of the temporal lobe. One of these genes selected for screening was LG11, described previously by Chernova and colleagues (5).

Mutation screening of LG11

In silico analysis of public databases showed that LG11 consists of 8 coding exons that were analyzed by direct sequencing of PCR products. In family ADLTE01, we found a 1bp deletion in exon 7 (c758delC, numbering from the start codon). This deletion changes the reading frame after its occurrence and introduces a new mutant-specific sequence of

![Figure 1](https://academic.oup.com/hmg/article-abstract/11/9/1119/2901635)
31 amino acids before the first in-frame stop codon is found. The change in the reading frame of the protein occurs at positions 862–927 of the cDNA, counting from the first codon. If translated, the mutated allele would produce a soluble protein of 283 amino acids instead of the 557 amino acids in the normal allele. This change segregated with the disease in all affected and obligate carriers of this family, whereas it was absent in 120 chromosomes from a healthy population with the same ethnic background (Fig. 1A, B). A second mutation was identified in family ADLTE03: a C→T transition found in position 1320 in exon 8. This change introduced a premature stop codon deleting the last 80 amino acids of the protein. In this nuclear family, the change also segregated with the disease and was not found in a population of similar ethnic background (Fig. 1C), strongly supporting LG11 as the gene causing the disease. We did not find evidence suggesting the existence of other mutations in the remaining families analyzed (see the Materials and Methods section for a description of the families collected).

**Alternative splicing of LG11**

As previously reported, LG11 is mainly expressed in brain tissue, with no particular differences amongst different areas within the central nervous system (5). In contrast with Chernova and co-workers (5), we observed three different species by northern blot (Fig. 2A): the main form of about 2.4 kb and two additional species – one of 1.6 kb and an additional band at about 3.2 kb. By PCR amplification from a cDNA library, we cloned the two major species and characterized them as alternatively spliced isoforms of the gene arising from the usage of a splicing site located within exon 8 (Fig. 2B; GenBank accession no. AF473548). This splicing event seems to be highly regulated, since the relative intensity of the two mRNA species in the northern (5) (Fig. 2A) is different in brain and in skeletal muscle. The spliced protein is 292 amino acids long, and the usage of the alternative splicing site changes the reading frame of the C-terminal part of the protein, modifying the last 12 amino acids prior to the

![Diagram](https://example.com/diagram.png)

**Figure 2.** Alternative splicing of LG11. (A) Northern blot of several tissues using IMAGE clone 178022. A strong signal is only observed in brain tissue, whereas a much weaker labeling of skeletal muscle can also be observed. (B) Alternative usage of a splicing site within exon 8. Capital letters represent exonic sequences, lower-case letters denote intronic sequences. Gray letters indicate the spliced-out portion of exon 8. (C) A graphical representation of LG11 and the effect of mutations in the translated protein. P01C258 and P03C262 indicate the epitopes of the polyclonal antibodies raised in rabbit (see the Materials and Methods section for further details).
Figure 3. Domain structure of LGI1 and KIAA1916. (A) Schematic representation of the domain structures of LGI1 and KIAA1916, showing the similarities in domain composition and organisation. Black boxes, signal peptide; Gray circles, N-terminal and C-terminal Cys-rich domains; light gray squares, LRR domains; white boxes, internal repeats 1 and 2. (B) Alignment of the two internal repeats found in LGI1, with their homologous counterparts in KIAA1916.

Figure 4. Expression of Lgi1 in adult mouse brain. Antibodies P01C258, which recognizes both the full-length and the spliced isoforms, and P03C262, which only recognizes the full-length isoform, were used to stain coronal sections of adult mouse brain (for further details, see the Materials and Methods section). (A) Coronal image of half brain showing staining with P01C258 in all areas. (B) A representative image of cortical staining (inset in A) with P01C258 in the cortex of adult mouse brain. All cortical layers show staining of neuronal bodies. (C) Close-up images of representative sections from hippocampus stained with antibodies P01C258 (top panel) and P03C262 (bottom panel). Note that only a few interneurons are labeled in the molecular layer. The same images were obtained for the equivalent antibodies raised in different animals against the same epitopes (see the Materials and Methods section). Scale bars indicate magnification.
first stop codon in frame. The nature of the largest band remains to be elucidated, although it might be due to the usage of alternative transcription or a polyadenylation signal.

Domain architecture of LGI1

In order to provide further insight into the putative function of LGI1, we performed an analysis of the primary structure of the protein using the SMART web-tool. In addition to the described signal peptide and the three leucine-rich repeats and flanking cysteine-rich domains (5), within the SMART web-tool, PROSPERO identified two copies of an internal repeated sequence of 136 and 130 amino acids at positions 226–361 and 420–549 respectively. Interestingly, the first of these repeats includes the region previously identified as encoding a transmembrane domain (5). To ascertain whether this repeated sequence was an exclusive feature of LGI1 or whether it was also found in other human proteins as well, we performed a similarity search using the Advanced BLAST2 Search Service (see the Materials and Methods section) and identified a putative paralogue of LGI1 encoded by KIAA1916 (GenBank accession no. AB067503) mapped to chromosome 4p15.1 (Fig. 3). In silico analysis of the currently available human genomic and EST sequences (Golden Path website, http://genom.ucsc.edu) suggested that the KIAA1916 gene is organized in 8 exons and that the corresponding putative polypeptide is 545 amino acids long. It has a similar size to LGI1, and shares significant similarity in domain architecture, exhibiting an identity of 57% and a similarity of 73% extended throughout the polypeptide molecule (Fig. 3).

Expression of LGI1

LGI1 cDNA was initially cloned based on its rearrangements in malignant brain tumors (5). In order to obtain further insight on the physiology of the gene product, we raised antibodies against regions of LGI1 conserved between human and mouse (6).

Immunohistochemistry performed on adult mouse brain sections showed Lgi1 to be widely expressed throughout the adult brain, mainly in neuronal cells, with little expression in glial cells (Fig. 4A). In this respect, there is an apparent higher level of expression of Lgi1 in several areas of the brain, such as the hippocampus and the piriform cortex, although this is likely caused by the higher density of cell bodies located in those areas in contrast with the neocortex. A parentel, all neurons are labeled; projection neurons are readily stained by both antibodies, whereas interneurons are infrequent (Fig. 4C). On the other hand, little labeling of glial cells can be observed throughout the brain – only in the white matter and other regions poor in neurons can a few cells be observed. Antibodies directed against the N-terminal part of LGI1 and against the C-terminal end of LGI1 yielded the same pattern of staining. In addition, polyclonal antibodies raised against the same antigens in different rabbits yielded comparable results (data not shown).

In mouse brain sections, in addition to cell bodies, we also observed punctuated staining characteristic of synaptic terminals (Fig. 5A). We also performed immunocytochemistry...
DISCUSSION

EPT is a mild form of epilepsy with an early onset, mild and scarce seizures, and excellent response to treatment. This genetic form of focal epilepsy, in the reported pedigrees, reaches penetrances of 70–80% (1,2). Here we report the identification of mutations in LGI1 in two unrelated Spanish families and provide further insight in the biology of this gene. Chernova and colleagues (3) previously identified LGI1 in glioblastoma cell lines as a tumor suppressor gene with a role in malignant brain tumors. Several candidate genes have been proposed in 10q23–q26 to account for some glioblastoma multiforme tumors (5). In the mutation carriers of the families analyzed here, neither the appearance of malignant glioblastoma nor an otherwise increased incidence of malignancies has been observed.

A signal peptide (positions 1–34), susceptible to direct LGI1 to the plasma membrane, and a single transmembrane domain have also been previously proposed by Chernova and collaborators (5). Although the prediction of a LGI1 transmembrane domain was obtained using computer programs that were state-of-the-art at the time they were employed (5), it was recently shown that they produce a rather high level of false-positive predictions (7). When we re-analyzed LGI1 using different computer programs shown to offer a lower false-positive rate (7), the significance values for the predicted transmembrane domain did not pass the program threshold. This suggests that LGI1 may enter the secretory pathway merely because of the presence of the signal peptide. The most remarkable feature of the protein is the presence of three leucine-rich repeats (LRR) in the predicted N-terminal domain of the protein, flanked by conserved cysteine-rich clusters. This type of repeat is the signature of a superfamily of proteins characterized by the presence of a relatively conserved sequence of 20–40 amino acids repeated a variable number of times. The functions of LRR-containing proteins are variable, and include signal transduction, cell adhesion and DNA repair, as well as extracellular matrix attachment (8) and plant steroid binding (9).

Recently, the mouse orthologue has been identified (6), although no other orthologues have been found in public databases in other model organisms, more distant in the evolutionary tree, such as Drosophila and Caenorhabditis elegans. Only weak similarities are found with other proteins harboring LRRs.

On the other hand, the presence of internal repeats also found in one other protein indicates that LGI1 could from part of a new family of proteins, with at least two members, combining both the LRR domains and the new type of repeats. The functional relationships between the two genes remains to be elucidated. The new type of repeat was submitted to Pfam, and will appear in the next release under the name EPT.

The observed mutations in EPT families introduce premature stop codons in LGI1 by generating a frameshift in the protein or by a genomic transition translated into a nonsense change in the amino acid sequence. Surprisingly, these remarkable alternations in the protein sequence are not accompanied by a severe phenotype, as is the case in other genetic disorders with similar genetic mutations (see for example 10). This might reflect the fact that the mutated alleles, if translated, will produce proteins that are only slightly different from their normal counterparts. The mutation observed in ADLTE01 introduces a premature stop codon in exon 7 that generates a protein closely resembling the naturally occurring spliced isoform of LGI1 (Fig. 2C). This mutation could exert its effect by altering the isoform ratio in the brain. As shown in Figure 2A, the abundance of each isoform is not the same in brain as it appears to be in skeletal muscle. The appearance of a premature stop codon in the mRNA encoding LGI1 could either deviate the mutant allele towards the RNA degradation machinery or produce a truncated protein closely resembling the spliced isoform. In any of those cases, the regulated ratio between both isoforms in the brain would be distorted. On the other hand, the second pedigree bears a mutation that also truncates the protein – though near its C-terminal end. In both cases, the functional relevance of the mutations is subtle enough for the disease to be relatively mild but important enough, with respect to its function, to account for the high penetrance in families with mutations.

Despite this ubiquitous presence of LGI1 throughout the brain, the staining pattern shown by the antibodies indicates that the labeling is mainly due to projection neurons, such as pyramidal neurons from the cortex, hippocampal neurons and Purkinje cells (data not shown), whereas little labeling is seen in other neuronal types, such as interneurons (Fig. 4C). The fact that not all neurons seem to express LGI1 brings up the question of which subpopulations are involved and how this relates to the disease process. In addition to this, although some labelling of glial cells is also observed, especially in white matter, these cell types do not seem to express LGI1 in significant numbers. This finding might be responsible for the obvious but hitherto unexplained lack of LGI1 expression in malignant glioblastomas, which consist predominantly of glial cells rather than neuronal cells. We have also shown that the protein is located in synaptic terminals (Fig. 5), suggesting a role in synaptic development, integrity or maintenance. In addition, in the absence of a putative transmembrane domain, LGI1 appears to be a protein associated with soluble compartments in neurons - the punctuate staining of the synaptic terminals and of cell bodies supporting this interpretation.

No other families show definite linkage to 10q24. Even though a few pedigrees provide some evidence for the genetic involvement of this region (11,12), linkage data are not conclusive in most cases. Our mutation-detection strategy by direct sequencing does not detect all coding mutations in the gene; for example, deletions of coding exons could not be detected. Nevertheless, it is also possible that another gene or genes account for the familial aggregation of lateral temporal epilepsy, especially in those kindreds responding to the
 MATERIALS AND METHODS

Human samples
We have analyzed a total of six index cases from Spanish pedigrees (ADLTE01–06), one German family (family G) and four pedigrees of Italian origin (pedigrees I1–I4). The index cases were clinically studied by at least one of us (J.J.P., J.G., J.F.M.M. or A.L.M. in Spain, V.F.M. or L.K. in Germany, and D.P., R.M., C.A.T., S.B. or G.A. in Italy). Details on ADLTE01, family G and I1 are available elsewhere (2,11,12). Clinical phenotypes were consistent with lateral temporal epilepsy with auditory features, with the exception of family I4, which shows a clinical picture consistent with a mesial origin of the disease. Informed consent for genetic analysis was obtained for all samples, and the ethical committees from the respective institutions involved in sample collection approved all the protocols. Family ADLTE02 corresponds to the ‘atypical’ form of EPT (J.J. Poza, R. Michelucci and collaborators, in preparation). Most of the collected pedigrees consist of small families with a reduced number of affected individuals, and showed a pattern of inheritance consistent with an autosomal dominant trait. When available, genetic analysis of 10q24 showed results that could not rule out this locus as causative for the disorder.

Sequencing of LGI1
The genomic structure of LGI1 was obtained from information derived from the human genome project. LGI1 cDNA sequence (GenBank accession no. AF055636) was blasted against the human genome sequence draft and a finished BAC (AL358154) was identified that contained the genomic sequence of LGI1. The intron/exon boundaries were deduced in silico from the alignment of those two sequences, and intronic primers were designed to amplify and sequence the coding exons. Primer sequences and PCR conditions used are shown in Table 1.

Mutation screening
PCR products were purified (Concert nucleic acid purification system, Invitrogen) and subjected to cycle sequencing using the BigDye v2.0 dye terminator kit and an ABI Prism 3100 Genetic Analyzer (Applied Biosystems).

Segregation and population analysis of mutations
In order to ascertain segregation of the mutations within each family, we designed an RFLP assay for each one. The mutation

<table>
<thead>
<tr>
<th>Exon</th>
<th>Forward</th>
<th>Reverse</th>
<th>Size (bp)</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>GGGGAGCTCCCTATGTGACCTG</td>
<td>TCCCTCTCCATGCCCTTCTAC</td>
<td>438</td>
<td>60°C</td>
</tr>
<tr>
<td>2</td>
<td>GCTAACAAGATACATAAAGAAG</td>
<td>GGCTACATCAATATACGCCC</td>
<td>219</td>
<td>48°C →</td>
</tr>
<tr>
<td>3</td>
<td>ACAATGTGACCTTCTACA</td>
<td>TTTGAGATCCTTTCTGATCT</td>
<td>171</td>
<td>48°C</td>
</tr>
<tr>
<td>4</td>
<td>CAGGACAAAGATCTCAGCTCTAG</td>
<td>ACAGGTGATCAACTGCTATG</td>
<td>197</td>
<td>60TD50</td>
</tr>
<tr>
<td>5</td>
<td>TCACGACGTTATACATCAC</td>
<td>AGGCTTCTCCCTGTAATG</td>
<td>172</td>
<td>50°C</td>
</tr>
<tr>
<td>6</td>
<td>CGTTGGGATTGCTCTGTTGAC</td>
<td>TTGGGAGATGCTCAGATTGATG</td>
<td>249</td>
<td>60TD50</td>
</tr>
<tr>
<td>7</td>
<td>GGAAGAAGAGTTATGCTCAC</td>
<td>CACTCATTTCTTCTGTTGC</td>
<td>287</td>
<td>50°C</td>
</tr>
<tr>
<td>8</td>
<td>TGGCCACACAACACTATCTCCTC</td>
<td>TCACTCCGCGGTATGTCATG</td>
<td>929</td>
<td>60TD50</td>
</tr>
</tbody>
</table>

aAfter the initial denaturation step, all PCR profiles were performed with 35 cycles, with 30 s at 94°C; 30 s at the indicated temperature and a 45 s elongation step, followed by 5 min of final elongation at 72°C. 60TD50 denotes a touchdown protocol where, after 4 cycles at 60°C, the annealing temperature decreases by 0.5°C/cycle until it reaches 50°C. Then the remaining cycles up to a total of 35 are performed at this lowest temperature. All PCR reactions were performed with a Taq polymerase from Qiagen.

The PCR requires the usage of the Q additive, which is part of the polymerise system.
in the ADLTE01 family, a 1 bp deletion, destroyed a Ddel site. Digestion of the amplified exon 7 with this enzyme at 37°C according to the manufacturer's instructions (MBI Fermentas) yields two fragments from the wild-type allele at 180 and 185 bp, whereas a single band at 365 bp appeared when the mutation is present.

On the other hand, the C→T transition in family ADLTE03 destroyed a NruI site. Digestion of the wild-type allele with NruI (MBI Fermentas) yields two fragments of 618 and 311 bp, whereas the undigested mutant allele yields a fragment of 929 bp.

To ascertain whether the changes found in the coding sequence of LG11 were pathogenic mutations or polymorphisms, we analyzed two populations of about 120 chromosomes each of similar ethnic background as each of the families on which the mutations were found. ADLTE01 is a family of Basque descent, and 60 unrelated healthy individuals were typed for the presence of the 1 bp deletion in exon 7. Similarly, ADLTE03 is a family originally from southern Spain, and we analyzed a sample of 60 healthy individuals from the south-east part of Spain when looking for the C→T transition in exon 8 of LG11.

Northern blot analysis

A nylon membrane containing mRNA from the tissues expressed was purchased from Clontech and hybridized with a probe obtained by random primer labeling of IMAGE clone 178022. Clone 178022 contains a 770 bp insert with sequence homologous to the 5’ end of LG11. The same probe was used to detect expression of LG11 in different brain areas (data not shown).

Sequence analysis

The primary sequence of LG11 was analyzed using the SMART server (Simple Modular Architecture Research Tool; http://smart.embl-heidelberg.de) for the annotation of the sequence (15,16). Analysis of the sequence to predict transmembrane domains was performed by TMHMM v2.0 (http://www.cbs.dtu.dk/services/TMHMM/). A search for possible palindromes was performed using the internal repeats detected by BLAST2 Search Service of EMBL. Analysis of human LG11 coding sequence was performed using the internal repeats detected by the SMART server (Simple Modular Architecture Research Tool; http://smart.embl-heidelberg.de) for the annotation of the sequence.

Antibody production

Polyclonal antibodies P01C258 and P03C262 were raised in rabbits against synthetic peptides coupled to KLH (DiverDrugs SL). Antibody P01C258 was raised against the peptide sequence found in the N-terminal region of LG11 (positions 201–220), EGPPYE KKRKINLSKSDKFD. On the other hand, antibody P03C262 was raised against the peptide DA-E KAKFVKFOELNVQAPR (positions 505–522) located in the C-terminal domain of LG11. Of these antibodies, P01C258 recognizes both the full-length protein and the spliced isoform, whereas P03C262 only recognizes the full-length isoform, since this epitope is located in the spliced-out region in the shorter isoform. Two animals were immunized with each peptide, and the results of the experiments shown here are equivalent with the antibodies obtained in the corresponding animals (data not shown). After taking preimmune serum from each animal, rabbits were immunized every three weeks with Freund's adjuvant (complete or incomplete, Sigma-Aldrich); after the third injection, the immunization level of the animals was assayed using the same immunogens in an ELISA assay. Three more immunizations took place before the animals were bledd. Immune sera were then obtained for each rabbit and the IgGs were purified by chromatography on a ProtA-Sepharose column (Amersham Biosciences).

Immunohistochemistry

Adult CD1 mice were anesthetized with chloral hydrate and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.3; PB). Coronal sections of thickness 30 μm through the brain were obtained with a vibratome, quenched in 3% hydrogen peroxide in PB, and blocked with 10% normal goat serum in PB (blocking buffer). Sections were incubated overnight with primary antibodies P01C258 and P03C262, and diluted at 1:400–1:1000 in blocking buffer, and detection was performed using the ABC-peroxidase method (Pierce) following the manufacturer's instructions. Preadsorption of primary antibodies with an excess of the corresponding non-KLH-coupled peptide prior to their addition to the sections resulted in complete absence of all labeling (data not shown).

Cell culture and immunocytochemistry

Neurons dissociated from cortices of E15.5 embryos were plated on poly-L-lysine and cultured for three days in BME supplemented with 5% horse serum and 5% fetal bovine serum. Cultures were fixed with 4% paraformaldehyde in PB and incubated with either mouse anti-MAP2 (1:100; Chemicon) or mouse anti-rab3 (1:200; Synaptic systems) and rabbit P01C258 antibody (1:400) in blocking buffer, followed by Cy2-conjugated anti-mouse, Cy3-conjugated anti-rabbit (Jackson Immunobiosciences) and DAPI (Sigma).

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