Two Novel Epilepsy-Linked Mutations Leading to a Loss of Function of LGII

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Background: Mutations in the leucine-rich, glioma-inactivated 1 (LGII) gene have been implicated in autosomal dominant lateral temporal epilepsy.

Objective: To describe the clinical and genetic findings in 2 families with autosomal dominant lateral temporal epilepsy and the functional consequences of 2 novel mutations in LGII.

Design: Clinical, genetic, and functional investigations.

Setting: University hospital.

Patients: Two French families with autosomal dominant lateral temporal epilepsy.

Main Outcome Measure: Mutation analysis.

Results: Two novel disease-linked mutations, p.Leu232Pro and c.431+1G>A, were identified in LGII. We demonstrated that the c.431+1G>A mutation causes the deletion of exons 3 and 4 of the LGII transcript and showed that the p.Leu232Pro mutation dramatically decreases secretion of the mutant protein by mammalian cells.

Conclusion: Our data indicate that LGII is a secreted protein and suggest that LGII-related epilepsy results from a loss of function.

Arch Neurol. 2007;64:217-222

Although temporal lobe epilepsy has been traditionally regarded as an acquired symptomatic disorder, evidence for familial temporal lobe epilepsy has been described. One form is autosomal dominant lateral temporal epilepsy (ADLTE) which is caused by mutations in the LGII (leucine-rich, glioma-inactivated 1) gene. The clinical hallmark of seizures in some patients is an auditory aura, which can be triggered by external noises. Ictal manifestations may include aphasic, visual, or pseudovertiginous symptoms, suggesting involvement of the lateral temporal lobe or adjacent cortical areas. Autosomal dominant lateral temporal epilepsy represents a benign clinical entity that is probably underestimated because the simple partial seizures are mild.

In 2002, the first LGII mutations were identified in families with ADLTE, followed by the description of many other mutations, including a de novo nonsense mutation. LGII encodes a neuronal protein also known as epitempin. The N-terminus has a signal peptide followed by 4½ leucine-rich repeats. The C-terminus consists of 7 tandem epilepsy-associated repeats (EARs) also identified in monogenic audiogenic seizures susceptibility protein (MASS1), which is mutated in a mouse line with audiogenic epilepsy. Although LGII was originally identified as a candidate tumor suppressor gene in gliomas, its role in brain tumors remains controversial. In addition, LGII is secreted to the extracellular environment by 293T cells. Unexpectedly, cytosolic LGII was recently shown to prevent inactivation of the presynaptic voltage-gated potassium channel subunit Kv1.1.

We report herein 2 novel LGII mutations, p.Leu232Pro and c.431+1G>A, in 2 families with ADLTE. Our functional investigations provide evidence that both mutations are deleterious and that in Chinese hamster ovary (CHO) and rat adrenal pheochromocytoma 12 (PC12) cells LGII is secreted to the extracellular environment.

METHODS

FAMILIES

Six families with partial epilepsy were selected for this study on the basis of the following characteristics: (1) At least 1 affected subject had auditory ictal symptoms. (2) Cerebral magnetic resonance imaging was normal in at least the proband. (3) There were affected individuals in several generations compatible with autosomal dominant inheritance. In family B, however, there were several loops of consanguinity that would also be compatible with a
recessive mode of transmission. Blood samples were obtained with informed consent, and genomic DNA was extracted using standard procedures.

**LGI1 MUTATION SCREENING**

Mutation analysis was performed in 1 index case from each of the 6 families with ADLTE by direct sequencing of all 8 LGI1-coding exons and flanking intronic splice sites. Primer sequences for the polymerase chain reaction (PCR) are available from us on request. Polymerase chain reaction products were sequenced using a sequence detection system (Big Dye Terminator Cycle, ABI PRISM; Applied Biosystems, Norwalk, Conn).

**REVERSE TRANSCRIPTION AND PCR**

We isolated total RNA from lymphoblasts of patient II-3 and from transfected CHO cells (RNasey Miniprep; Qiagen, Studio City, Calif.). The corresponding complementary DNA (cDNA) was obtained by oligo-dT–primed reverse transcription (Thermoscript; Invitrogen, San Diego, Calif.). Exons 1 to 5 were then amplified by PCR, followed by a nested PCR because of the low abundance of LGI1 messenger RNA (mRNA) in lymphoblasts.

**CELL CULTURE AND TRANSFECTION**

Using full-length mouse wild-type LGI1 cDNA with a FLAG tag at the N-terminus, LGI1L1232P and LGI1E383A were generated using a commercially available kit (Quickchange; Stratagene, La Jolla, Calif.). CHO and PC12 cells were cultured in Dulbecco modified Eagle medium containing 10% fetal bovine serum, 10 U/mL of penicillin G sodium, and 10 µg/mL of streptomycin sulfate. All transient transfections were performed using a commercially available kit (Transfection kit; Hyclone, Logan, Utah). The corresponding complementary DNA (cDNA) was obtained by oligo-dT–primed reverse transcription (Thermoscript; Invitrogen, San Diego, Calif.). Exons 1 to 5 were then amplified by PCR, followed by a nested PCR because of the low abundance of LGI1 messenger RNA (mRNA) in lymphoblasts.

**RESULTS**

**CLINICAL DESCRIPTION OF THE FAMILIES WITH LGI1 MUTATIONS**

All clinical examinations were performed at the Hôpital de la Pitie–Salpetriere, Paris, France. Family A, of French origin, had 3 affected subjects, all with normal birth and psychomotor development. None had febrile seizures. The proband (I-2) was a 64-year-old right-handed man. Partial seizures began at age 11 years. He described simple partial seizures lasting 30 seconds to 1 minute consisting of bilateral buzzing sounds during which speech comprehension was impaired. No triggering factor was reported. Generalized seizures were rare. Partial seizures were frequent during the first years of the disease. At the time of the study, the patient (taking a daily regimen of 100 mg of phenytoin sodium, 10 mg of phenobarbital sodium, and 2 mg of clonazepam) had been seizure free for 4 years. His daughter (II-3), 31 years old and right-handed, had chronic depression. Epilepsy began at age 22 years with generalized tonic-clonic seizures and probable complex partial seizures with swallowing automatisms. She described no auditory symptoms. At adolescence, she started to experience dreamy states with déjà vu and déjà vécu anxiety, thoracic pressure, and sometimes sensory aphasia. She has remained seizure free since beginning treatment with antiepileptic medication (valproic acid). Her deceased sister (II-4) started to have seizures at age 24 years consisting of generalized tonic-clonic seizures sometimes preceded by auditory symptoms described as bilateral buzzing sounds. Treatment with vigabatrin and valproic acid did not control the seizures, but adherence to the treatment regimen was irregular. She was depressed and committed suicide in 1996 at age 31 years. The deceased brother was also depressed and committed suicide at age 20 years. He may have had generalized tonic-clonic seizures during adolescence.

Family B was of Algerian origin. Seven members were affected, but only 3 of them could be examined for the study. All 3 subjects had normal births and psychomotor development. None had febrile seizures. The proband (III-13) was a 39-year-old left-handed man. The disease began when he was 9 years old, manifested by generalized tonic-clonic seizures and partial seizures that started with the impression of hearing whistling, followed by anxiety, nausea, and a rising sensation within the body. More than 50% of his seizures were triggered by sounds, such as telephones ringing. Taking a daily regimen of 500 mg of primidone and 100 mg of phenobarbital sodium, the seizures became rare (1-2 per year). His sister (III-14) was 33 years old and right-handed. Her partial seizures began at age 10 years, manifesting as a buzzing sensation in the ears followed by loss of consciousness with gestural and verbal automatisms. Some seizures were triggered by high-pitched voices. Several antiepileptic drugs were used with little effect (carbamazepine, valproic acid, phenobarbital, and topiramate). Despite drug treatment, she continued to have several partial seizures per month, some with secondary generalization. When she was 26 years old, their 56-year-old mother (II-3) began to have complex partial seizures that were preceded by auditory symptoms and a rising sensation within the body. She has been seizure free taking a regimen of phenobarbital for the last 20 years.

**IDENTIFICATION OF NOVEL LGI1 MUTATIONS**

We identified 2 novel LGI1 mutations in families A and B. A c.431 + 1G>A substitution located in the almost invariant donor splicing site of intron 5 was found in family A (Figure 1A). The mutation was present in the heterozygous state in the 2 available patients (Figure 1B). It was not found in 170 European control subjects.

The consequences of the c.431 + 1G>A mutation were explored at the mRNA level. Reverse-transcriptase PCR was performed on mRNA isolated from lymphoblasts of patient II-3 and from an unrelated control subject. Polymerase chain reaction amplification of the region between exons 1 and 3 produced a single band of 398 base pairs (bp) in the control sample and an additional band of 254 bp in
the affected individual (Figure 1C). DNA sequencing showed that the c.431 + 1G>A mutation caused exons 3 and 4 to be skipped in the LGII transcript, confirming that the deletion conserves the reading frame, the predicted protein should lack 48 amino acids.

In family B, a c.695t>C substitution was detected in exon 7 causing the leucine at position 232 to be replaced by a proline (p.Leu232Pro/L232P). The nucleotide change cosegregated in the heterozygous state with epilepsy in the 3 available patients (Figure 2A and B). It was not found in 170 European and 84 North African control subjects. Leucine 232 is located within the second EAR and is highly conserved throughout evolution (Figure 2C).

p.Leu232Pro MUTATION DRAMATICALLY DECREASES LGII SECRETION

We then investigated the functional consequences of the p.Leu232Pro mutation in mammalian cells. We first examined the expression of exogenous LGI1WT in the cell lysates and in the serum-free media of transiently transfected CHO cells by WB analysis. An antibody raised against the C-terminus of LGI1 recognized a band at approximately 64 kDa corresponding to FLAG LGI1WT in the cell lysate (Figure 3A, lane 1). However, most LGI1WT was found in the culture medium (Figure 3A, lane 5), indicating that LGI1 is secreted by CHO cells.

Next, we investigated the functional consequences of LGI1L232P and compared them with those of the previously reported LGI1E383A mutant.2 In the lysates, the levels of LGI1WT, LGI1L232P, and LGI1E383A were similar (Figure 3A, lanes 3 and 7). However, in the culture medium only LGI1WT was detected under the exposure condition used. Similar results were obtained using the anti-FLAG antibody that recognizes the N-terminus of the protein (data not shown).

The absence of mutant LGI1 in the culture media was not due to inefficient transcription or mRNA instability because mRNA of all 3 constructs was similarly reverse transcribed and amplified (Figure 3A, middle panel).

Because protein trafficking in CHO and neuronal cells may differ, we explored the secretion of LGI1 in the neuronlike PC12 cell line. Figure 3B shows that LGI1WT, but not LGI1L232P or LGI1E383A, was secreted by PC12 cells. These experiments confirmed that LGI1 is a secreted protein. They also demonstrated that the p.Leu232Pro and p.Glu383Ala mutations markedly reduce protein stability or secretion.
LGI1<sup>L232P</sup> AND LGI1<sup>E383A</sup> ARE NOT PREFERENTIALLY DEGRATED BY THE PROTEASOME PATHWAY

We hypothesized that the reduced extracellular levels of LGI1 mutants might have resulted from accelerated degradation by the ubiquitin-proteasome system, the main cellular pathway for the degradation of proteins. To verify this hypothesis, transfected CHO cells were grown in the presence of the proteasome inhibitor epoxomicin. Western blot analysis of cell lysates showed that LGI1<sup>WT</sup>, LGI1<sup>L232P</sup>, and LGI1<sup>E383A</sup> accumulated in the cells, indicating that the proteasome inhibitor prevented their degradation. However, it did not rescue the levels of secreted mutant proteins (Figure 4). Although wild-type and mutant LGI1 are normally degraded by the proteasome pathway, this does not explain the decreased secretion of the mutant proteins.

COMMENT

In this study, we identified 2 novel disease-linked mutations (c.431+1G>A and p.Leu232Pro) in 2 of 6 families with ADLTE. The frequency of the mutations was similar to that in other investigations in which 30% to 50% of families with ADLTE had LGI1 mutations. The clinical characteristics of our patients were consistent with previous descriptions of families with ADLTE (Table). Patient II-3 in family A had unusual ictal symptoms with déjà vu and déjà vécu suggesting involvement of the medial temporal lobe. In family A, all patients with epilepsy had psychiatric disturbances, but we could not determine whether they are caused by the same mutation as epilepsy. The course of the disease was variable within families in terms of the presence of a triggering factor, type of seizure, and pharmacosensitivity.
We also demonstrated that the c.431 + 1G>A mutation alters mRNA splicing and leads to the in-frame deletion of exons 3 and 4 of the LGI1 transcript. The predicted truncated protein lacks 48 amino acids in the leucine-rich repeat region, which might affect interactions between LGI1 and other proteins. However, we cannot exclude that the mutant mRNA is degraded and that haploinsufficiency of LGI1 causes the disease.

We also studied the functional consequences of the p.Leu232Pro mutation in transfected mammalian cells and compared them with those of the previously described p.Glu383Ala mutation known to decrease secretion of the protein. Both mutations are located in an EAR motif; the p.Leu232Pro mutation is located in the second EAR of the protein, whereas p.Glu383Ala affects a glutamic acid in the fourth EAR of the protein. In CHO cell cultures, we showed that the p.Leu232Pro mutation prevented the secretion of LGI1, as demonstrated by Western blot with the anti-LGI1 antibody SC-9583 that detected LGI1 in the lysates and the culture media of transfected CHO cells treated for 16 hours with 1µM epoxomicin.

Table. Clinical Characteristics of the 2 Families With Autosomal Dominant Lateral Temporal Epilepsy With LGI1 Mutations

<table>
<thead>
<tr>
<th>Family A</th>
<th>Patient No.</th>
<th>Sex</th>
<th>Age at Onset, y</th>
<th>Auditory Symptoms</th>
<th>Other Symptoms</th>
<th>Interictal Electroencephalogram</th>
<th>Magnetic Resonance Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>I-2/M</td>
<td>11</td>
<td>M</td>
<td></td>
<td>Yes</td>
<td>Aphasia</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>II-3/F</td>
<td>Adolescence</td>
<td>No</td>
<td></td>
<td>No</td>
<td>Aphasia and dreamy states</td>
<td>Right temporal slow waves and temporo-occipital spikes</td>
<td>Normal</td>
</tr>
<tr>
<td>II-4/F</td>
<td>24</td>
<td>F</td>
<td></td>
<td>Yes</td>
<td>None</td>
<td>Normal</td>
<td>Normal</td>
</tr>
</tbody>
</table>

Table. Clinical Characteristics of the 2 Families With Autosomal Dominant Lateral Temporal Epilepsy With LGI1 Mutations

<table>
<thead>
<tr>
<th>Family B</th>
<th>Patient No.</th>
<th>Sex</th>
<th>Age at Onset, y</th>
<th>Auditory Symptoms</th>
<th>Other Symptoms</th>
<th>Interictal Electroencephalogram</th>
<th>Magnetic Resonance Imaging</th>
</tr>
</thead>
<tbody>
<tr>
<td>III-13/M</td>
<td>9</td>
<td>M</td>
<td></td>
<td>Yes</td>
<td>Rising sensation within the body, nausea</td>
<td>Left frontotemporal slow waves and spikes</td>
<td>Normal</td>
</tr>
<tr>
<td>III-14/F</td>
<td>10</td>
<td>F</td>
<td></td>
<td>Yes</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>III-2/F</td>
<td>26</td>
<td>F</td>
<td></td>
<td>Yes</td>
<td>None</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

Abbreviation: NA, not available.

*All patients had partial seizures and generalized (secondary) tonic-clonic seizures.
the protein into the culture medium, suggesting that a loss of function underlies the pathogenesis of LGII-mediated epilepsy. Because we could not restore secretion by inhibiting the proteasomal degradation pathway, lack of secretion rather than loss of the protein seems to be responsible. However, we cannot exclude that mutant LGII was degraded by extracellular proteases or was associated with the cell membrane and was not recovered with the detergents used.

These experiments provide evidence that overexpressed LGII is secreted by CHO and PC12 cells, confirming previous observations in 293T cells. Our findings suggest that the c.431+1G>A and p.Leu232Pro mutations lead to loss of function by different mechanisms, with the c.431+1G>A mutation leading to a truncated or missing protein and the p.Leu232Pro mutation leading to loss of the protein from the extracellular compartment where it appears to act.

The pathogenic mechanism for LGII-mediated epilepsy has not yet been elucidated. LGII was reported to modulate the properties of a potassium channel through protein-protein interactions with the intracellular domains and to be secreted in heterologous cells. This apparent paradox could be explained by the recent identification of 2 isoforms of LGII in the brain, one of which might be secreted, whereas the other might act within the cell, as is the case for the alternatively spliced forms of dectin-1 (dectin-1E isoform is cytosolic, whereas dectin-1A is secreted).

In addition, the observation that LGII is also expressed in areas that do not express voltage-gated potassium channel subunit Kv1.13 suggests that LGII has functions in addition to the modulation of this channel. Recently, Fukata and collaborators showed that ADAM22, a transmembrane protein, serves as a receptor for LGII. In addition, application of LGII in hippocampal slices enhances AMPA alpha-amino-3-hydroxy-5-isoxazolepropionate receptor–mediated synaptic transmission.

Accepted for Publication: October 12, 2006.

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Financial Disclosure: None reported.

Funding/Support: This study was supported in part by the Association de Recherche sur la Génétique des Épilepsies and sponsored by Sanofi-Aventis.

Role of the Sponsor: The sponsor of the study had no role in the design and conduct of the study, in the collection, analysis, and interpretation of the data, or in the preparation, review, or approval of the manuscript.

Acknowledgment: We are grateful to the patients and their families. We would like to thank Mark Ruberg, PhD, for critical reading of the manuscript, Jeffrey Noebels, MD, PhD, for providing the LGII cDNA clone, and Fabienne Picard, MD, for referring the 3 families with ADLTE.

REFERENCES


**Correction**

Misspellings and Incorrect First Name. In the article titled “Two Novel Epilepsy-Linked Mutations Leading to a Loss of Function in LGII” by Chabrol et al, published in the February issue of the Archives (2007;64:217-222) INSERM was misspelled in the “Author Affiliations” and “Correspondence” sections on pages 217 and 222, respectively. Also in the “Author Affiliations” and “Correspondence” sections, the parenthetical information should have read as follows: “(Institut National de la Sante´ et de la Recherche Me´dicale, Unite´ Mixte de Re- cherche 679).” The beginning of the second sentence of the “Acknowledgment” section on page 222 should have read as follows: “We would like to thank Merle Ruberg, PhD, for critical reading of the manuscript...”