A Novel Loss-of-Function LGI1 Mutation Linked to Autosomal Dominant Lateral Temporal Epilepsy

Pasquale Striano, MD; Arturo de Falco, MD; Erica Diani, BSc; Giorgia Bovo, BSc; Sandra Furlan; Libero Vitiello, PhD; Federica Pinardi, MD; Salvatore Striano, MD; Roberto Michelucci, MD; Fabrizio Antonio de Falco, MD; Carlo Nobile, PhD

**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.

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**METHODS**

This Italian family had 8 affected members in 3 generations (Figure 1). All of the participants gave written, informed consent under a protocol approved by the local ethics committee. A comprehensive medical history was obtained through a personal interview of each participating subject, and information about the occurrence and frequency of seizures, age at onset, presence and nature of aura and semiology, and possible risk factors was collected. Seizure types were classified according to the Partial Seizure Symptom Definitions. Family members who did not report seizures were specifically asked about auditory and other sen-
sory (aphasic, visual, vertiginous, psychic, epigastric) phenomena. A detailed neurologic examination and prolonged EEG recording were also performed in each subject.

We extracted DNA from blood by standard methods, and LGII exons were amplified by polymerase chain reaction as described previously.3 Sequencing of polymerase chain reaction products was performed using the Big Dye Terminator Cycle Sequencing kit (ABI PRISM; Applied Biosystems, Foster City, California) and an ABI3730 automated sequencer (Applied Biosystems).

A cell transfection assay was performed as described in detail previously.2 Briefly, LGII wild-type or LGII 365T>A expression constructs containing a Flag peptide in frame with the LGII complementary DNA sequence were transfected into human embryonic kidney 293 cells. Twenty-four hours after the beginning of transfection, the cells were washed twice and then refed with serum-free medium Opti-MEM (Invitrogen Corp, Carlsbad, California). After about 20 hours, cells were lysed and the medium was collected and concentrated about 40 times using Centricron YM30 concentrators (Millipore Corp, Billerica, Massachusetts). Aliquots of cell lysates and concentrated medium were loaded on a sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel and analyzed by Western blot using the anti-Lgi1 antibody ab30868 (Abcam Inc, Cambridge, Massachusetts) or the anti-Flag antibody F7425 (Sigma-Aldrich Co, St Louis, Missouri).

MOLECULAR GENETIC ANALYSIS

Sequencing of LGII exons in the proband III:8 revealed a heterozygous c.365T>A mutation (numbering from the start codon) (Figure 2A) in exon 4, giving rise to an isoleucine to lysine substitution at position 122 of the protein sequence (Ile122Lys). The mutation was present in the other available affected family members (II:1, III:1, and III:2) but not in subject III:3 (with a febrile seizure only) and in 130 unrelated healthy control subjects of Italian ancestry. The Ile122 residue is conserved in many species, including mouse, rat, chicken, and zebrafish (data not shown). Replacement of this hydrophobic amino acid with the charged lysine residue likely disrupts the structure and hampers the function of the mutated protein.

CELL TRANSFECTION ASSAY

To ascertain the functional consequences of the Ile122Lys mutation, we transfected LGII wild-type and LGII 365T>A complementary DNA into human embryonic kidney 293 cells, which do not express endogenous LGII, and analyzed the proteins produced by these cells using immunoblot. Both cell lysates and concentrated (about 40 times) serum-free media were analyzed using anti-Lgi1 and anti-Flag antibodies (see the “Methods” section). The Lgi1 wild-type protein was detected mostly in the medium of transfected cells, although some signal was retained in the cell lysate, whereas the mutated protein was detected only in the cell lysate (Figure 2B). Thus, the mutated Lgi1 protein carrying the Ile122Lys point mutation is not secreted
from transfected cells, as previously shown for other single amino acid substitutions.8,11,12

**Table. Clinical Details of the Investigated Family Members**

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Febrile Seizures</th>
<th>Seizure Semiology</th>
<th>Age at Onset, y</th>
<th>Tonic-Clonic Seizures (Age, y)</th>
<th>Interictal EEG Results</th>
<th>MRI Results</th>
<th>Outcome</th>
<th>AED Therapy</th>
<th>LGI1 Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>II:1/M/52</td>
<td>No</td>
<td>Rhythmic echoes in ears getting louder (SPS)</td>
<td>24</td>
<td>Yes (24)</td>
<td>Normal</td>
<td>ND</td>
<td>SF</td>
<td>Phenytoin</td>
<td>Yes</td>
</tr>
<tr>
<td>III:1/F/36</td>
<td>No</td>
<td>Uniformed sounds or voices, déjà vu (SPS)</td>
<td>25</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
<td>Monthly SPS</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>III:2/F/33</td>
<td>No</td>
<td>Uniformed sounds, déjà vu (SPS)</td>
<td>14</td>
<td>No</td>
<td>Normal</td>
<td>Normal</td>
<td>Monthly SPS</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>III:3/F/28</td>
<td>Yes</td>
<td>NA</td>
<td>14</td>
<td>No</td>
<td>Normal</td>
<td>No</td>
<td>SF</td>
<td>Carbamazepine</td>
<td>No</td>
</tr>
<tr>
<td>III:8/F/28</td>
<td>No</td>
<td>Buzzing in left ear, loss of contact (SPS, CPS)</td>
<td>26</td>
<td>Yes (28)</td>
<td>Left temporal sharp waves</td>
<td>Normal</td>
<td>SF</td>
<td>Yes</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: AED, antiepileptic drug; CPS, complex partial seizures; EEG, electroencephalography; MRI, magnetic resonance imaging; NA, not applicable; ND, not done; SF, seizure-free; SPS, simple partial seizures.

**COMMENT**

Identification of ADLTE is clinically important because this form of focal epilepsy generally has a favorable prognosis with good response to antiepileptic therapy. Because the clinical diagnosis of ADLTE is based mainly on the presence of an auditory aura, which may be elusive in some patients or families, testing for mutations in LGI1 is important to confirm diagnosis of ADLTE, especially in families with only a few patients available. Also, detection of LGI1 mutations in familial cases with lateral temporal epilepsy could contribute—together with the clinical data—to avoiding long presurgical studies and preventing unnecessary surgery.

The predominant feature of the family described here was the occurrence of simple partial seizures with exclusive or predominant auditory symptoms, suggesting an onset in the lateral temporal lobe cortex. Interestingly, 2 subjects (III:1 and III:2) with déjà vu reported a strong auditory component of their sensation. Complex partial seizures with loss of contact were reported by 1 patient only. Tonic-clonic seizures preceded or not preceded by aura occurred in 2 patients. In all of the affected members, epilepsy was very mild in severity, with low seizure frequency also in patients not receiving antiepileptic drugs. One family member had a febrile seizure during infancy and lacked the LGI1 mutation, confirming that febrile seizures are not part of the ADLTE phenotype, at least in families with documented LGI1 mutations.

The causative role of the LGI1 Ile122Lys mutation is supported by its segregation with ADLTE in affected members, its absence in control chromosomes, its negative effect on secretion of the mutated protein, and the evolutionary conservation of the Ile122 residue. The Ile122 amino acid is part of the hydrophobic core of the second LRR repeat and therefore is important for proper folding of the LRR domain. Several other structural missense mutations affecting the LRR protein region (Cys42Arg, Cys46Arg, Cys200Arg, and Leu154Pro) have been found in families with aphasic as well as auditory auras. Because no affected members of our family experienced auras with aphasic symptoms, our findings do not support any correlation between mutations involving structural LRR residues and auras with an aphasic component. However, a larger number of ADLTE kindreds with LGI1 mutations is needed to correlate genotypes and phenotypes reliably.

LGI1 defines a new class of epilepsy genes because it differs structurally from ion channel genes implicated in other inherited forms of epilepsy. The mechanism by which LGI1 mutations determine epilepsy remains unclear. Senechal and coworkers8 showed that the Lg1 protein produced in transfected nonneuronal cells is secreted and that mutations either truncating the protein or replacing single amino acids hamper the secretion process. These findings suggest that Lg1 may function as a ligand in the extracellular or synaptic environment.

Recent work suggests that the Lg1 protein may serve as a ligand for the postsynaptic receptor ADAM22, a model implying secretion of Lg1 into the synaptic cleft.14 The identification of ADLTE-causing mutations that hamper secretion of the Lg1 protein, like the mutation described in this article, lends further

![Figure 2](https://example.com/figure2.png)

**Figure 2.** Molecular genetic analysis and cell transfection assay. A, Original sequence tracings used to detect the disease allele. Arrow indicates the variant allele. B, Immunoblot analysis of transfected human embryonic kidney 293 cells. Cell lysates (L) and concentrated media (M) of human embryonic kidney 293 cells transfected with LGI1 wild-type Flag or LGI1 365T>A Flag expression constructs or with empty expression vector were analyzed by Western blot using either an anti-Lg1 or anti-Flag antibody. Arrows indicate the position of the 60-kDa Lg1-Flag fusion protein detected by these antibodies.
support to this functional model. Additional characterization of the LGII mutational spectrum and of the functional effect of mutations will help to elucidate the normal Lgi1 function and its role in epileptogenesis.

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Correspondence: Carlo Nobile, PhD, Istituto di Neuroscienze del CNR, Sezione di Padova, Dipartimento di Scienze Biomediche Sperimentali, Università di Padova, viale G. Colombo 3, 35121 Padova, Italy (nobile@bio.unipd.it).

Author Contributions: Dr Nobile had full access to all of the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Drs P. Striano and A. de Falco contributed equally to the study. Study concept and design: P. Striano, A. de Falco, Vitiello, S. Striano, Michelucci, and Nobile. Acquisition of data: P. Striano, A. de Falco, Furlan, Vitiello, Pinardi, F. A. de Falco, and Nobile. Analysis and interpretation of data: P. Striano, A. de Falco, Bovo, and Nobile. Drafting of the manuscript: P. Striano, A. de Falco, Furlan, Vitiello, Pinardi, F. A. de Falco, and Nobile. Critical revision of the manuscript for important intellectual content: A. de Falco, Vitiello, S. Striano, Michelucci, F. A. de Falco, and Nobile. Obtained funding: Nobile. Administrative, technical, and material support: Diani, Bovo, Furlan, and Pinardi. Study supervision: P. Striano, A. de Falco, S. Striano, Michelucci, F. A. de Falco, and Nobile. Financial Disclosure: None reported.
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REFERENCES