

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

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Background: Mutations responsible for autosomal dominant lateral temporal epilepsy have been found in the leucine-rich, glioma-inactivated 1 (*LGII*) 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 *LGII* 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 *LGII* 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 *LGII* hamper secretion of the Lgi1 protein, thereby precluding its normal function.

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AUTOSOMAL DOMINANT LATERAL temporal epilepsy (ADLTE) (OMIM600512), or autosomal dominant partial epilepsy with auditory features, is an inherited epileptic syndrome characterized by focal seizures with ictal auditory phenomena or other symptoms suggesting a lateral temporal lobe onset of discharge.¹⁻³ Seizures may be triggered by environmental sounds or noises, and secondarily generalized tonic-clonic seizures (TCSs) are almost invariably present but occur sporadically. The evolution of the condition is relatively benign because seizures are usually well controlled with standard antiepileptic drugs.^{3,4} Conventional brain magnetic resonance imaging results are normal and electroencephalograms (EEGs) frequently show mild temporal abnormalities. Mutations in the leucine-rich, glioma-inactivated 1 (*LGII*) gene (GenBank 9211), also named *Epitempin*, are found in about 50% of the families, compatible with autosomal dominant inheritance with reduced penetrance and suggesting that ADLTE is genetically heterogeneous.³⁻⁵ The *LGII* gene is mainly expressed in brain tissues and shows no homology with known ion channel genes. It encodes a protein whose pre-

dicted structure consists of 4 LRR repeats⁶ in the N-terminal portion and 7 EPTP repeats⁷ in the C-terminal region, each domain defining a distinct family of proteins exerting a variety of functions. The function of *LGII* is unclear. Recent in vitro experiments have shown that the Lgi1 protein produced by transfected cells is secreted.^{8,9}

Here we describe the clinical and genetic findings of a previously unreported kindred in which affected individuals show ADLTE phenotype due to a novel *LGII* mutation.

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.¹⁰ Family members who did not report seizures were specifically asked about auditory and other sen-

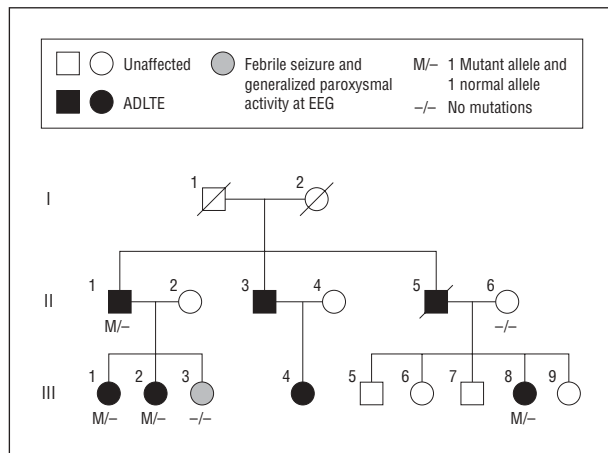


Figure 1. Simplified pedigree of the family. ADLTE indicates autosomal dominant lateral temporal epilepsy; EEG, electroencephalography.

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.⁹ 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 Centricon 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).

RESULTS

CASE DESCRIPTIONS

The family pedigree is shown in Figure 1 and the clinical features of the affected members are summarized in the **Table**. The proband (patient III:8), a 28-year-old, right-handed woman, had a single TCS and a 2-year history of monthly simple partial seizures characterized by buzzing in her left ear and déjà vu; on some occasions these episodes were followed by loss of contact lasting about 30 seconds. She denied any specific and unusual modality of induction. Neurologic examination results, 1.5-T magnetic resonance imaging results, and auditory-evoked potentials were normal. Prolonged video-EEG monitoring after sleep deprivation disclosed rare, slow, sharp waves over the left temporal regions. She began receiving carbamazepine (400 mg/d) and did not report fur-

ther seizures at 8 months' follow-up. Patient II:1 was a 51-year-old, right-handed man with a history of simple partial seizures that started at age 24 years and are characterized by rhythmic echoes in both ears progressively getting louder; sometimes a secondarily generalized TCS could ensue. Sleep deprivation and stress could facilitate seizures. His neurologic examination, prolonged EEG monitoring, and brain magnetic resonance imaging results were normal. Therapy with phenytoin (200 mg/d) started at age 30 years allowed complete seizure control. At age 40 years, voluntary withdrawal of therapy resulted in a TCS. However, no further seizures occurred after resumption of the phenytoin treatment. Patients III:1 and III:2 were right-handed women aged 36 and 33 years, respectively, with seizures characterized by déjà vu sensation with a marked auditory component or simple ictal auditory symptoms (unformed sounds, voices) that started at ages 25 and 14 years, respectively. No TCSs or specific triggering stimuli were reported. Their neurologic examination results, 1.5-T magnetic resonance imaging results, and prolonged video-EEG recording results after sleep deprivation were unremarkable. Although simple partial seizures continued on a monthly basis, the patients refused to start any therapy. Patient II:5 died at age 42 years due to a pancreatic carcinoma and had TCSs preceded by "sounds in his ears" from adolescence. Patient III:3 had a single febrile seizure at age 6 months.

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

Table. Clinical Details of the Investigated Family Members

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

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.

from transfected cells, as previously shown for other single amino acid substitutions.^{8,11,12}

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 *LGII* is important to confirm diagnosis of ADLTE, especially in families with only a few patients available. Also, detection of *LGII* 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 *LGII* mutation, confirming that febrile seizures are not part of the ADLTE phenotype, at least in families with documented *LGII* mutations.^{3,4}

The causative role of the *LGII* 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,⁵ Cys46Arg,^{13,14} Cys200Arg,³ and Leu154Pro¹⁵) have been found in families with aphasic as well as auditory auras. Because no affected members of our family

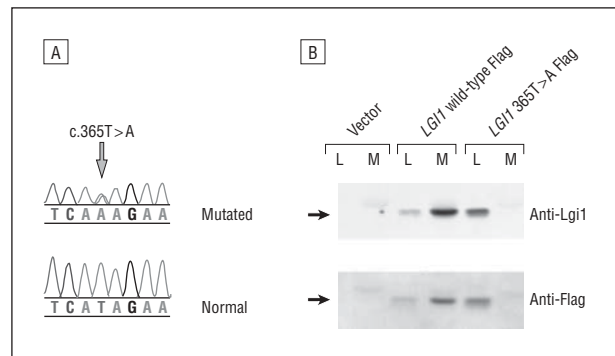


Figure 2. Molecular genetic analysis and cell transfection assay. A, Original sequence tracings used to detect the disease allele. Arrow indicates the variant allele. B, Immunoblot analysis of transfected human embryonic kidney 293 cells. Cell lysates (L) and concentrated media (M) of human embryonic kidney 293 cells transfected with *LGII* wild-type Flag or *LGII* 365T>A Flag expression constructs or with empty expression vector were analyzed by Western blot using either an anti-Lgi1 or anti-Flag antibody. Arrows indicate the position of the 60-kDa Lgi1-Flag fusion protein detected by these antibodies.

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 *LGII* mutations is needed to correlate genotypes and phenotypes reliably.

LGII 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 *LGII* mutations determine epilepsy remains unclear. Senechal and coworkers⁸ showed that the Lgi1 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 Lgi1 may function as a ligand in the extracellular or synaptic environment.

Recent work suggests that the Lgi1 protein may serve as a ligand for the postsynaptic receptor ADAM22, a model implying secretion of Lgi1 into the synaptic cleft.¹⁶ The identification of ADLTE-causing mutations that hamper secretion of the Lgi1 protein, like the mutation described in this article, lends further

support to this functional model. Additional characterization of the *LG11* 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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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, Diani, 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, Diani, Bovo, Furlan, Pinardi, 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.

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