Epilepsia, **44**(10):1289–1297, 2003
Blackwell Publishing, Inc.
© 2003 International League Against Epilepsy

Clinical Research

Autosomal Dominant Lateral Temporal Epilepsy: Clinical Spectrum, New Epitempin Mutations, and Genetic Heterogeneity in Seven European Families

*Roberto Michelucci, †Juan Jose Poza, ‡Vito Sofia, §Maria Rita de Feo, ||Simona Binelli, ¶Francesca Bisulli, **Evan Scudellaro, ††Barbara Simionati, ††Rosanna Zimbello, *Giuseppe d'Orsi, ‡‡Daniela Passarelli, ¶Patrizia Avoni, ||Giuliano Avanzini, ¶Paolo Tinuper, ‡Roberto Biondi, ††Giorgio Valle, §§Victor F. Mautner, ||||Ulrich Stephani, *Carlo Alberto Tassinari, ¶¶Nicholas K. Moschonas, ***Reiner Siebert, †Adolpho Lopez de Munain, †††Jordi Perez-Tur, and **Carlo Nobile

**Dipartimento di Neuroscienze, Divisione di Neurologia, Ospedale Bellaria e Università di Bologna, Bologna, Italy; †Department of Neurology, Hospital Donostia, San Sebastián, Basque Country, Spain; ‡Dipartimento di Neuroscienze, Università di Catania, Catania; §Dipartimento di Scienze Neurologiche, Università "La Sapienza," Roma; ||Istituto Nazionale Neurologico "Carlo Besta," Milano; ¶Dipartimento di Scienze Neurologiche, Istituto di Clinica Neurologica, Università di Bologna, Bologna; **CNR-Istituto di Neuroscienze-Sezione di Biologia e Fisiopatologia Neuromuscolare, and ††CRIBI, Università di Padova, Padova; ‡‡Ospedale Infermi, Faenza, Italy; §§Department of Neurology, Klinikum Hamburg Nord, and ||||Neuropediatric Clinic, University of Kiel, Kiel, Germany; ¶¶Institute of Molecular Biology and Biotechnology, Heraklion, Greece; ***Institute of Human Genetics, University Hospital Kiel, Kiel, Germany; and †††Unitat de Genetica Molecular, Institut de Biomedicina de Valencia (CSIC), Valencia, Spain*

Summary: *Purpose:* To describe the clinical and genetic findings of seven additional pedigrees with autosomal dominant lateral temporal epilepsy (ADLTE).

Methods: A personal and family history was obtained from each affected and unaffected member, along with a physical and neurologic examination. Routine and sleep EEGs, computed tomography (CT), or magnetic resonance imaging (MRI) were performed in almost all the patients. DNAs from family members were typed with several microsatellite markers localized on either side of LGI1 at 10q24 and screened for LGI1 mutations.

Results: The seven families included a total of 34 affected individuals (10 deceased). The age at onset ranged between 8 and 50 years (average, 22 years). Twenty-six patients had clear-cut focal (elementary, complex, or secondarily generalized) seizures, characterized by prominent auditory auras in 68% of the cases. Less frequent ictal symptoms were visual, psychic, or aphasic seizures, the latter occurring in isolation in one family. The attacks were rare and well controlled by antiepileptic drug treatment but recurred after drug discontinuation. Interictal EEGs were usually unrevealing. MRI or CT scans were negative. Analysis of LGI1/Epitempin exons failed to show mutations in three pedigrees. Linkage analysis strongly suggested exclusion of linkage in one of these families. We found two novel missense mutations, a T→C substitution in exon 6 at position 598, and a T→A transition in exon 8 at position 1295, the latter being detected in a family with aphasic seizures.

Conclusions: Our data confirm the inclusion of aphasic seizures within the ADLTE clinical spectrum, suggest the existence of locus heterogeneity in ADLTE, and provide new familial cases with LGI1 missense mutations associated with the disease. **Key Words:** Autosomal dominant lateral temporal epilepsy—Auditory features—LGI1/Epitempin gene—Missense mutations.

Autosomal dominant lateral temporal epilepsy (ADLTE), otherwise described as autosomal dominant partial epilepsy with auditory features (ADPEAF), is a recently recognized epileptic condition characterized by autosomal dominant transmission, an average age at onset in late adolescence, focal and secondarily generalized tonic–clonic seizures with typical auditory auras and/or symptoms suggesting a lateral temporal onset, absence of any brain structural abnormality, and benign evolution.

Accepted May 23, 2003.
Address correspondence and reprint requests to Dr. R. Michelucci at Department of Neurosciences, Division of Neurology, Bellaria Hospital, via Altura, 3, 40139 Bologna, Italy. E-mail: Roberto.Michelucci@ausl.bo.it

Since the first description by Ottman et al. in 1995 (1), this condition was found to be linked to a locus on chromosome 10q24; in the following years, additional large pedigrees provided evidence for the same genetic localization (2,3), and haplotype analyses of small families were consistent with an identical linkage to 10q24 (4–6). These findings suggested a genetic homogeneity for ADLTE and contributed to focusing the efforts to identify the responsible gene.

Recently, the disease-causing gene for ADLTE was identified independently by us (7) and other groups (8,9): it is the leucine-rich gene, glioma-inactivated 1 (LGI1), formerly described by Chernova et al. (10) and now also named Epitempin (7), whose mutations often introduce premature stop codons, resulting in truncated protein products. LGI1 was cloned based on its rearrangements in the T98G glioblastoma multiforme cell line (10). It is expressed mainly in brain tissues and has been found to be downregulated in many high-grade glioma tumors (10). The predicted protein structure of LGI1 consists, in the N-terminal portion, of three leucine-rich repeats (LRRs) flanked on both sides by typical cysteine-rich repeat sequences (11), and, in the C-terminal region, of seven copies of the newly identified EPTP repeat (12). LRRs are widespread among different classes of intra- and extracellular proteins and often mediate interactions to other proteins (11). The predicted structure of EPTP repeats is compatible with that of beta-propeller domains, which are involved in a variety of functions, including protein–protein interactions (13).

Although all the ADLTE families reported so far display mutations in it (7–9,14), it is not known whether LGI1 is the only gene involved in ADLTE. At this stage, as many ADLTE families as possible should be tested for mutations in LGI1/Epitempin and typed with closely flanking markers to detect novel mutations and to verify whether ADLTE is genetically heterogeneous, as are other familial idiopathic epileptic syndromes (15).

Here we report the extensive clinical and genetic findings in seven previously unreported European families, collected as part of a collaborative project of the European consortium for the study of ADLTE.

PATIENTS AND METHODS

Clinical data collection

After publication of our original European pedigrees with ADLTE (2,4,5), we collected seven additional families in Italy and Spain, also with the aid of the local chapters of the International League Against Epilepsy. Families were selected on the basis of the following clinical criteria: autosomal dominant inheritance, seizures whose semiology clearly indicated a lateral temporal lobe onset (not only auditory features), absence of neurologic signs, and absence of any known structural brain pathology or etiology.

Each affected or presumptively affected individual was interviewed and directly examined by one of us (R.M., J.P., A.L.M., C.A.T., V.S., M.R.dF., S.B., F.B.), either at the hospital or during a visit to the patient's home, possibly in the presence of as many family members as possible to ensure complete ascertainment of seizures or other relevant family information. A personal and family history was obtained from each affected and unaffected member along with a physical and neurologic examination. Sometimes unaffected members were interviewed only by phone to exclude occurrence of seizures. Medical records describing results of neurophysiologic, neuroimaging, and history data were collected whenever possible to supplement the clinical visits.

Patients were considered affected in the presence of two or more unequivocal unprovoked seizures; if doubt could arise about additional etiologic factors (cerebral insults, metabolic conditions, etc.), the case was kept apart and defined as symptomatic or probably symptomatic.

Routine EEGs with 16 electrodes positioned according to the international 10-20 system with bipolar and referential montages were performed in almost all the patients and in a few unaffected members. Sleep EEGs during afternoon nap also were obtained in most affected subjects. A computed tomographic (CT) or magnetic resonance imaging (MRI) scan was available in almost all the patients.

Genotype determination and linkage analysis

Blood samples were collected from family members after informed consent was obtained and DNA was extracted by a standard phenol method. DNAs were typed with several microsatellite markers, including D10S185, D10S1680, and D10S574, localized on either side of LGI1 at 10q24. Polymerase chain reaction (PCR) amplification of microsatellite markers was performed as described (16) with fluorescently labeled primers in a thermal cycler (MJ Research, Waltham, MA, U.S.A.). PCR products were fractionated on an ABI3100 (16 capillaries) apparatus (Applied Biosystems, Foster City, CA, U.S.A.). Linkage analysis was performed by using the MLINK program of the LINKAGE package version 5.2; LOD score values were calculated by assuming a disease-allele frequency of 0.0001, penetrance of 0.70, and autosomal dominant inheritance.

LGI1 mutation screening

PCR amplifications of genomic DNA fragments containing each LGI1 exon were performed as described in Morante-Redolat et al. (7). Alternatively, the primers shown in Table 1 were used under the following conditions: 4 min at 94°C followed by 30 cycles (1 min at 94°C, 1 min at 62°C, 1 min at 72°C). Sequencing of PCR products was performed by using the Big Dye Terminator Cycle Sequencing kit (ABI PRISM, Applied Biosystems)

TABLE 1. *LGI1 exon-specific primers*

Exon	Forward	Reverse	Product size (bp)
1	GGTGGACTCCTATGTGACCTG	TCTCTCTCCATGCCCTTCTAC	408
2	CCTGTAGCCGATTCATTTCTCT	GCAAACAAACCCATCTACCTCT	402
3+4	TCTGCATAACTAACACTGTAGCAGAC	ATAAAGTAGGTGCATTAACCACAGG	465
5	AGGCTGGAAATGACAAAAGAGA	CCAGGCTTCCTTGTTAATGACT	239
6	TCTGAGCCCAAAGTGAATGAG	TTTGAGGTGGAATGATGATGAG	365
7	TCATGTGCAGGAACTGATATTTTT	CATTCCCCTATACCACTCATCTTT	467
8a	TGTTTACATGCTCCAAAAGAGGA	CTCCATGTTAGGAATGTCAGTTTG	512
8b	ATTTATCAGTGGAACAAAGCAACA	ATCCGGGTCATGTACTGTAGAAA	501

and an ABI377 automatic sequencer (Applied Biosystems).

PCR-restriction fragment link polymorphism (RFLP) analysis of exon 6 was performed with *Mvn*I in family I-1 and control DNAs. The 598T > C mutation introduced a single *Mvn*I restriction site in the PCR fragment amplified with the primers for exon 6 (Table 1). Screening of control DNAs for the 1295T > A mutation was done by allele-specific oligonucleotide (ASO) PCR by using primers (5′ > 3′): ATTCCTAACATGGAGGATGA; TTTATTTGAAGAGGCTGGAA.

The primers shown in Table 1 also were used to perform semiquantitative PCR analysis of DNAs from patients and healthy members of families in which no mutations could be detected by sequencing. The most appropriate number of replication rounds was determined preliminarily by amplifying 50 and 100 ng of genomic DNA for a number of cycles varying from 17 to 21 under the conditions described earlier. We then chose to PCR amplify 100 ng of DNA from one patient of each family for 19 cycles. PCR products were fractionated by electrophoresis on a 5% acrylamide gel and visualized by silver staining.

RESULTS

Clinical description of the families

As a whole, we collected seven families (five from Italy and two from Spain) fulfilling the ADLTE inclusion criteria and including a total of 95 individuals (excluding spouses), of whom 34 were considered definitely affected (10 deceased). The clinical, EEG, and neuroimaging findings of each family are described in detail in Table 2, and individual pedigrees are shown in Fig. 1.

General data

Affected family members were 18 male (35% of all males) and 16 female subjects (33% of all females), suggesting that both sexes were almost equally involved.

The age at onset ranged between 8 and 50 years, with a mean of 21.8 years. In most cases, however, the disease began in adolescence or early adulthood. Interestingly, in family I-3, all the six affected individuals began their seizure history at the same age (i.e., ~20 years).

No history was found of significant personal antecedent or pathology in any affected member. In particular, no patients had febrile seizures. One patient died of Hodgkin lymphoma.

Seizure semiology and evolution

On the basis of a detailed epileptologic history, the 34 patients were found to have either clear-cut focal (elementary, complex, or secondarily generalized) seizures (26 cases) or tonic–clonic seizures of unknown origin (eight cases, mostly deceased). Of the 26 patients with focal (partial) seizures, 11 had only secondarily generalized tonic–clonic seizures, and 15 had both secondarily generalized tonic–clonic seizures and elementary (six cases) or complex (nine cases) partial seizures.

The focal seizures (either elementary, complex, or secondarily generalized) were characterized by prominent auditory auras in 23 (68%) patients. Two patients with auditory symptoms also reported visual features during the same attacks or in isolation. Psychic symptoms such as depersonalization, pleasure, or fear followed the auditory auras in three cases. Difficulty in speaking or loss of speech comprehension was strictly linked with the auditory symptoms in two patients. Interestingly, the three patients belonging to family I-2 had either aphasic seizures without auditory components (two cases) or complex partial seizures preceded by an ill-defined feeling of "fixed gaze" and "general malaise" (one case). Aphasic seizures were described as "inability to understand spoken or written words and phrases" with intact consciousness.

The auditory symptoms were usually reported as an elementary and unformed sound (such as buzzing, ringing, or humming); one patient described structured voices, and three further individuals reported sudden loss of the surrounding noise (as if they had become deaf).

Specific triggering stimuli of seizures were reported by four patients, three of them belonging to family I-1: sudden noises (such as telephone ringing, slamming of doors, entering a noisy room), listening to the radio, or answering the phone could precipitate the seizures, which also occurred spontaneously. In another family (S-2), nonspecific factors, such as somnolence and alcohol consumption, were sometimes responsible for seizure occurrence.

TABLE 2. *Clinical findings of the families*

Clinical findings	FAM S-1	FAM S-2	FAM I-1	FAM I-2	FAM I-3	FAM I-4	FAM I-5
No. of patients (a/d)	5/6	3/0	4/0	3/0	3/3	3/1	3/0
Male/female	6/5	1/2	3/1	3/0	1/5	2/2	2/1
Age at onset (yr) (range)	15–20	15–23	18–50	12–19	18–20	8–30	22–46
Seizure types							
PS (no.)		1	3	3	2	3	3
SGTC (no.)	10	2	3	3	2	3	3
UOTC (no.)	1	1	1		4	1	
Ictal symptoms							
Auditory (no.)	10	2	3		2	3	3
Visual (no.)							2[a]
Aphasic (no.)			1[a]	2			1[a]
Other (no.)	2[a]		1[a]	1			2[a]
Triggering stimuli no.	None	"Some"	3	None	None	1	None
Type		Somnolence, alcohol consumption	Sudden noises; telephone			Listening to radio; noisy rooms	
EEG (no.)	3	3	3	3	3	2	3
Ictal	—	—	—	—	—	—	—
Interictal (findings, no.)	Normal (3)	Normal (3)	Mild temp. slow abn. (3)	Mild temp. slow abn. (3)	Mild temp. epilept (1) and slow (1) abn.	Mild temp. slow abn. (2)	Rare bil. Temp. sharp waves with left predom. (1), rare left temp. sharp waves (1), bursts of diffuse slow waves (1)
CT/MRI	3	3	3	3	3	2	3
Normal (no.)	3	3	3	3	2	2	2
Abnormal (no.)	—	—	—	—	Mild temp horn asymmetry (1)	—	Mild atrophy of the right hemisphere (1)

a, alive; d, deceased; PS, partial seizure; SGTC, secondarily generalized tonic–clonic seizure; UOTC, tonic–clonic seizure of unknown origin (whether primary or secondarily generalized); CT, computed tomography; MRI, magnetic resonance imaging; abn, abnormalities; temp, temporal; bil, bilateral; epilept, epileptiform.

[a] These symptoms were secondary components of the auras.

Seizure frequency was generally low: secondarily generalized tonic–clonic seizures were sporadic or occurred once to twice per year, whereas elementary or complex partial seizures were more frequent, occurring several times per month to 2 to 5 times per year. However, given the mild intensity and "unusual" features of the minor seizures, these could continue unrecognized for several years.

Once the medical therapy was commenced, however, the seizures were promptly controlled in all cases; any drug, including carbamazepine (CBZ), phenobarbital (PB), and phenytoin (PHT), was usually effective, sometimes at low doses.

Three patients interrupted the treatment after a few years of seizure freedom: seizure recurrence was constant and prompted the resumption of therapy.

Neurophysiology

Routine EEGs were available in 21 living patients, and sleep EEGs after sleep deprivation were obtained in 15 cases. The recordings were usually unrevealing, showing completely normal findings (eight cases) or mild temporal slow (10 cases) or paroxysmal (three cases) abnormalities. No ictal EEG tracing was available.

Neuroimaging

An MRI scan was available in 15 patients, and a CT scan, in an additional six individuals. The examinations were normal in all except two: one patient showed mild atrophy of the right hemisphere, and another individual (belonging to a different family) displayed mild temporal horn asymmetry.

Genetic findings

Mutation analysis of LGI1/Epitempin

Mutation analysis of LGI1/Epitempin coding exons and flanking intronic splice sites, performed by direct sequencing, failed to show mutations in families I-3, I-4, and I-5, as well as in two families reported previously by our group (4,5).

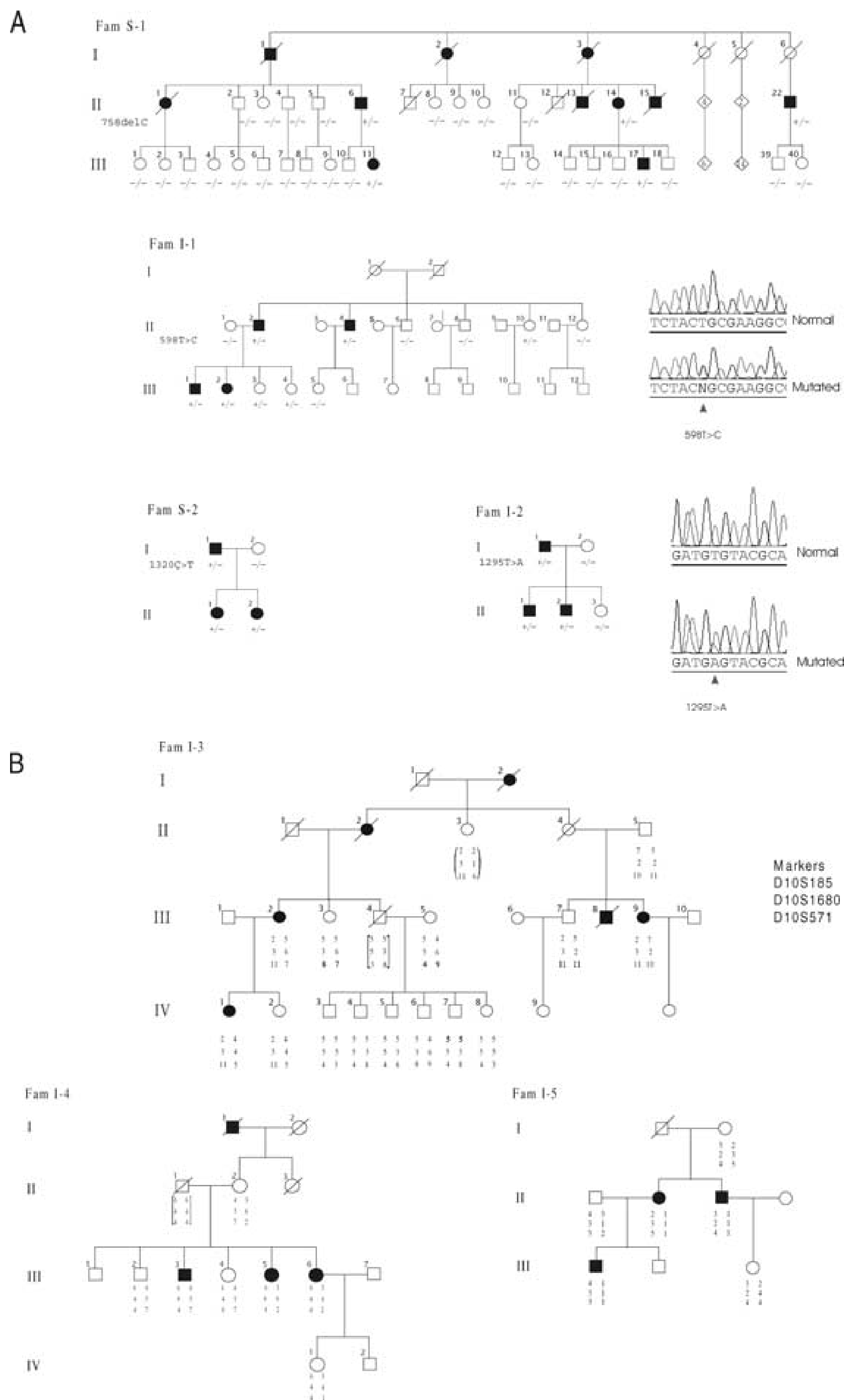

FIG. 1. Pedigree structures of families with ADLTE and segregation of microsatellite alleles or putative mutations. A: Pedigrees found mutated in LGI1. Individuals with no mutations are denoted by −/−, whereas those who carried one mutant and one normal allele, by +/−. The type of missense mutation is indicated for each family. Original sequence tracings used to detect disease alleles are shown to the right of pedigrees I-1 and I-2. Variant alleles, red arrows. Mutations 758delC and 1320C > T have been described elsewhere (6). Solid circles and squares, female and male subjects with partial epilepsy; open symbols, healthy family members. B: Pedigrees showing no mutations in LGI1 with allelic haplotypes for the microsatellite markers indicated (alleles are in the same order). D10S185 lies about 330 kb centromeric to LGI1; D10S1680 and D10S571, ~85 and 1,600 kb telomeric to LGI1, respectively. The highest 2-point LOD scores at a recombination frequency of 0.0 were –3.54 (family I-4, all markers), 0.20 (family I-5, marker D10185), and 0.36 (family I-3, marker D10S571). Parentheses, alleles of unknown phase; brackets, haplotypes reconstructed from those of wives and offspring.

In the Italian family I-1, a T → C substitution was detected in exon 6 at position 598 from the start codon (598T→C; Fig. 1A). The nucleotide change was found to cosegregate with the disease in this family and, in addition, was detected in three healthy subjects (II:10, III:3, and III:4), who also carried the marker haplotype associated with the disease (not shown). Because of the variable age at onset of the disease in this family (18–50 years), subjects III:3 and III:IV, who are in their twenties, are at risk for the disease, whereas II:10, who is 79, can be regarded as a healthy carrier. The 598T→C substitution created a new *Mvn*I restriction site, which was used in a PCR-based assay to confirm the occurrence of the mutation in the family and to show its absence in 102 Italian control individuals (data not shown). At the protein level, the 598T→C mutation causes the cysteine at position 200 to be replaced by an arginine (C200R). The C200 residue occurs at the third position in a cluster of four highly conserved cysteines ($CxCx_{20}Cx_{20}C$; x can be any amino acid) flanking the LRR array on the C-terminal side. In extracellular proteins, both the C- and N-terminal cysteine-rich regions are integral components of the LRR domain (11).

Another missense mutation, a T→A transition occurring in exon 8 at position 1295 from the start codon (1295T→A; Fig. 1A), was detected in the affected members of the Italian family I-2, in which no patients experienced auditory seizures (see earlier). This mutation was not found in 102 controls. At the protein level, it causes the valine at position 432 to be replaced by a glutamic acid residue (V432E). The V432 residue occurs in the fifth EPTP repeat (12) and is conserved in mouse and rat.

The Spanish family S-2 had a premature stop codon mutation caused by a C→T transition at position 1320 in exon 8, which has been already reported elsewhere (7). The other Spanish family S-1 was found to carry the same mutation (758delC in exon 7) as detected in family ADLTE01 reported in Morante Redolat et al. (7) and was identified in the same geographic area.

Finally, to detect possible exon-spanning deletions in families in which no point mutations were shown by direct sequencing of exons, we performed semiquantitative PCR analysis of individual exons. No deletion mutations were identified by this technique (see, for example, Fig. 2).

Linkage analysis

We performed linkage analysis of the pedigrees without LGI1 mutations for microsatellites D10S185, D10S1680, and D10S571, closely flanking LGI1. In family I-4, in which affected individuals show discordant haplotypes (Fig. 1B), we obtained a 2-point LOD score value of −3.54 at a recombination frequency of 0.0 for each marker, formally excluding linkage to the LGI1 locus in this family. Linkage analysis of the remaining families, I-3 and I-5, gave inconclusive results.

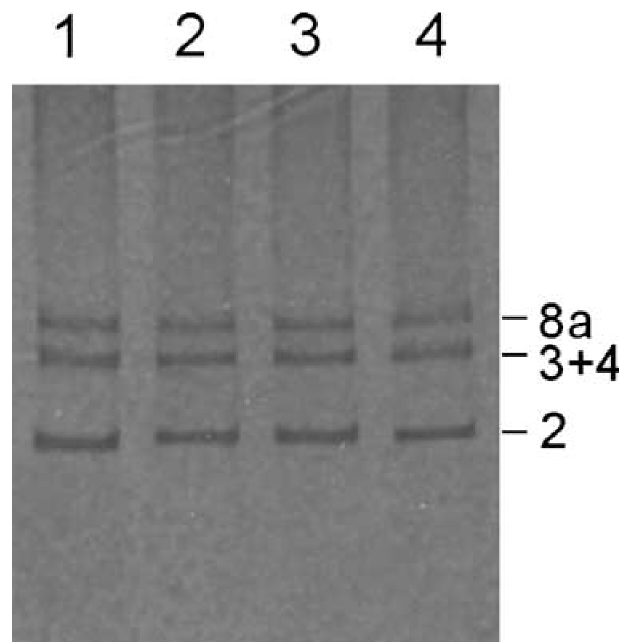

FIG. 2. Example of semiquantitative polymerase chain reaction analysis of patient DNAs from families with no mutations detectable by sequencing. Patients were from families 1, I-5; 2, I-3; 3, I-4; 4, control DNA. The exons analyzed in this experiment are indicated on the right.

DISCUSSION

We describe the electroclinical and genetic findings of seven new families with ADLTE collected in Italy and Spain. The analysis of clinical data concerning 34 patients belonging to the seven families allowed us to delineate a phenotype closely resembling previous descriptions of the condition (1–6,14,17–19) (Table 3): absence of any relevant personal history or associated illnesses, no sex predominance, a mean age at onset of 21.6 years with a range spanning from infancy to adulthood, rare focal seizures (elementary, complex, or secondarily generalized) with typical auditory features sometimes triggered by sudden external noises, complete control of seizures by conventional therapy, absence of any neurologic abnormality, inconstant and mild temporal EEG abnormalities, and normal neuroimaging.

In our series, tonic–clonic seizures (either secondarily generalized or of unknown generalized or focal origin) were the only seizure type in 57% of the cases and were associated with elementary or complex partial seizures in the remaining patients. Therefore each patient of our families had tonic–clonic seizures, which could occur at any time of the day. This high incidence of tonic–clonic seizures also was reported in previous published families (Table 3).

TABLE 3. *Overall clinical data of the published ADLTE families and the present series*

Author	Family (no.)	Patient (no.)	M/F	Age at onset (aver.) (range)	SGTC no. (%)	UOTC (no.)	PS (no.)	Aud no. (%)	Vis. (no.)	Aph. (no.)	Other (no.)	Trig. Stim (no.)
Ottman et al. (1)	1	11	8/3	12 (8–19)	10 (91)	1	9	6 (54)	2	0	8	1
Poza et al. (2)	1	19[a]	12/7	24 (11–40)	9 (81)	1	8	4 (36)	6	0	2	"some"
Michelucci et al. (5)	1	6[b]	3/3	8 (6–12)	3 (100)	0	0	3 (100)	0	0	0	0
Mautner et al. (4)	1	9	4/5	18 (11–50)	8 (89)	0	0	8 (89)	0	0	0	0
Kanemoto et al. (19)	1	5	2/3	14 (9–20)	4 (80)	0	5	0 (0)	0	5	0	3
Ikeda et al. (18)	1	3	2/1	15 (15–16)	3 (100)	3	3	3 (100)	0	1	1	1
Brodtkorb et al. (3)	1	17[c]	8/9	18 (4–42)	5 (42)	7	11	5 (42)	0	8	4	7
Winawer et al. (6)	4	19	Unkn	16 (9–30)	15 (79)	4	4	14 (74)	2	1	12	2
Pizzuti et al. (14)	1	5[d]	1/4	13 (9–15)	3 (75)	1	4	3 (75)	0	2	2	0
Present series	7	34	18/16	22 (8–50)	26 (77)	8	15	23 (68)	2	4	3	7
Total	19	128	58/51	18 (4–50)	86 (77)	25	59	69 (62)	12	21	32	21

M/F, male/female; SGTC, secondarily generalized tonic–clonic seizure; UOTC, tonic–clonic seizure of unknown origin (whether partial or generalized); PS, partial seizure (elementary or complex); Aud, auditory; Vis, visual; Aph, aphasic; Other, other ictal symptoms; Trig, stim, triggering stimuli.

[a]Sufficient clinical information available only in 11.
[b]Sufficient clinical information available only in three.
[c]Sufficient clinical information available only in 12.
[d]Sufficient clinical information available only in four.

Detailed semiology of auras disclosed a high prevalence (68%) of auditory auras; other types of ictal symptoms, usually following the auditory features, were rare and included psychic (8.5%), visual (6%), or aphasic (11%) components. Two of the three patients belonging to the I-2 family reported aphasic seizures without any auditory feature. Brodtkorb et al. (3) described a Norwegian family linked to 10q24, and later shown to bear a LGI1 mutation (9), in which several members had aphasic seizures usually associated with elementary auditory hallucinations. In our patients, the semiology of ictal aphasic symptoms (loss of comprehension) suggested that the epileptic discharges involved the temporal language areas.

Several ADLTE families have been screened for mutations in LGI1 by various groups, and mutations have been found in all cases. The five American families reported by Kalachikov et al. (8) all showed mutations affecting either the coding sequence (four cases, three frameshift and one missense mutations) or a splice site (one case). Three additional pedigrees reported by other groups (9,14,20) had missense mutations. Overall, we found mutations in five of 10 European ADLTE families analyzed, including the three pedigrees published previously (2,4,5). No sequence alterations, including exon-spanning deletions, were detected in the remaining five pedigrees. Although it cannot be ruled out the possibility that mutations may occur in the regulatory or other noncoding regions of LGI1, our data suggest that at least another gene is involved in ADLTE. This notion is supported by (a) the negative LOD score (–3.54) obtained in family I-4 with markers closely flanking LGI1; (b) the high proportion (50%) of families in our series with no apparent mutations in LGI1; and (c) the existence of families in which partial epilepsy with prominent auditory symptoms is transmitted independently from the 10q24 locus with a low penetrance (21). The comparison of clinical data between families mutated in LGI1 and those with no mutations did not disclose any overt difference. This makes it reasonable to suppose that the additional gene(s) involved in ADLTE could be structurally and/or functionally related to LGI1. Three genes, provisionally termed LGI2, LGI3, and LGI4 (9,12,22), encoding proteins structurally similar to LGI1 (each with three LRRs and seven EPTP motifs) expressed in adult human and mouse brain (23) (T. Sarafidou, N.K. Moschonas, et al., unpublished data), are good candidates for ADLTE and will be screened in families with no mutations in LGI1/Epitempin.

The majority of the LGI1/Epitempin mutations so far reported resulted in truncated, presumably nonfunctional, protein products (7,8). Only three missense mutations have been fully described. One of these results in the replacement of one of the four conserved cysteines flanking the LRR array on the N-terminal side with an arginine (C46R) (9); another one determines the substitution of a glutamic acid residue with an alanine at position 383 (E383A) in the C-terminal region of the protein (8); and a third mutation, described very recently (14), causes a leucine-to-arginine change at position 26 (L26R) in the signal peptide sequence.

As in the C46R mutation (9), the C200R mutation reported in this article causes a conserved cysteine to be replaced by an arginine. The affected cysteine found in our family, however, belongs to the cluster located C-terminal to the LRR repeats (see Fig. 3).

Conserved cysteines flanking LRR repeats probably form intra- or intermolecular disulfide bonds in extracellular LRR proteins (24). Evidence suggesting an important functional role of cysteine clusters has been reported: the N-terminal cluster has been mapped as a ligand-binding site of the human platelet glycoprotein Ib (25); conversely,

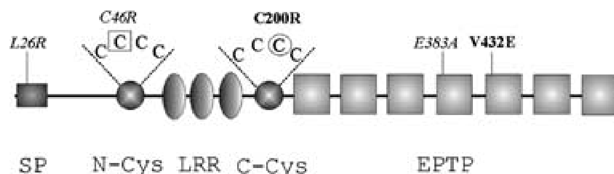

FIG. 3. Schematic representation of the Lgl1/Epitempin domains and localization of missense mutations. Different geometric symbols are assigned to the various protein domain types indicated by the abbreviations: SP, signal peptide; LRR, leucine-rich repeat region; N-Cys, cysteine-rich region N-terminal to LRR; C-Cys, cysteine-rich region C-terminal to LRR; EPTP, C-terminal repeats as defined by Staub et al. (10). Missense mutations in bold are described in this article; those in italics have been reported by other groups (see text). The four conserved cysteines composing the N- and C-Cys regions are shown. Mutations affecting the circled and boxed Cs have been described in this article and by Gu et al. (12), respectively.

several mutations altering the function of the *Drosophila* Toll gene have been found to affect the cysteines of the cluster C-terminal to the LRRs (26). The C200R mutation shown in this article and the C46R mutation described previously (9) strongly support the importance of both cysteine-rich clusters for the correct function of the Lgi1/Epitempin protein and their involvement, when mutated, in the mechanisms ultimately leading to seizures.

The C-terminal region of the Lgi1/Epitempin protein consists of seven EPTP repeats (12), a structure reminiscent of the beta-propeller domain (13). The V432E mutation reported in this article affects a conserved valine residue in the fifth EPTP repeat, whereas the E383A mutation reported by Kalachikov et al. (8) affects a glutamic acid residue that is part of fourth EPTP repeat (see Fig. 3). Both these missense mutations entail a remarkable modification of the electrical charge at the corresponding position, which likely alters the normal function of the implicated EPTP repeat.

Considering all the missense mutations revealed so far, no obvious phenotype/genotype correlation appears to emerge; in particular, aphasic features of seizures were associated with mutations occurring either in the LRR domain (C46R) or in the EPTP repeat region (V432E), as were auditory seizures.

The condition we refer to as ADLTE was originally reported under the heading of ADPEAF by Ottman et al. (1), due to the peculiar prevalence of auditory symptoms as part of the seizures. Although it is clear that the auditory features represent the most frequent type of aura in this condition and are particularly useful for confirming the correct diagnosis, other ictal symptoms (such as visual auras) suggesting a seizure onset from the lateral portion of the temporal lobe have been described. In particular, the evidence that patients with familial aphasic seizures have mutations in the same gene, as shown in the Norwegian family (9) and our I-2 pedigree, further widens the clinical spectrum of this condition and supports the usefulness of the term ADLTE rather than ADPEAF.

Acknowledgment: This work was supported by the Genetic Commission of the Italian League Against Epilepsy (LICE) and in part by Telethon-Italy (grant GGP02339 to R.M. and C.N.) and MIUR-Italy (grant 2000 to C.A.T.). Work at the JP-T laboratory was funded through a grant from the Spanish Ministry of Science and Technology (SAF2002-00060). Studies on tumor-suppressor genes in 10q24 and LGI1 changes in neurologic diseases are supported by the Deutsche Krebshilfe and the Hensel-Stiftung Kiel, respectively. We also thank the members of the families described for their generous participation, physicians (Drs. L. Rivera and A. Calvirossi) who helped us to enlarge some pedigrees, and Mrs. C. Giardini for editing the manuscript.

REFERENCES

1. Ottman R, Risch N, Hauser WA, et al. Localization of a gene for partial epilepsy to chromosome 10q. *Nat Genet* 1995;10:56–60.
2. Poza JJ, Sáenz A, Martínez-Gil A, et al. Autosomal dominant lateral temporal epilepsy: clinical and genetic study of a large Basque pedigree linked to chromosome 10q. *Ann Neurol* 1999;45:182–8.
3. Brodtkorb E, Gu W, Nakken KO, et al. Familial temporal lobe epilepsy with aphasic seizures and linkage to chromosome 10q22-q24. *Epilepsia* 2002;43:228–35.
4. Mautner VF, Lindenau M, Gottesleben A, et al. Supporting evidence of a gene for partial epilepsy on 10q. *Neurogenetics* 2000;3:31–4.
5. Michelucci R, Passarelli D, Pitzalis S, et al. Autosomal dominant partial epilepsy with auditory features: description of a new family. *Epilepsia* 2000;41:967–70
6. Winaver MR, Martinelli Boneschi F, Barker-Cummings C, et al. Four new families with autosomal dominant partial epilepsy with auditory features: clinical description and linkage to chromosome 10q24. *Epilepsia* 2002;43:60–7.
7. Morante-Redolat JM, Gorostidi-Pagola A, Piquer-Sirerol S, et al. Mutations in the LGI1/Epitempin gene on 10q24 cause autosomal dominant lateral temporal epilepsy. *Hum Mol Genet* 2002;11:1119–28.
8. Kalachikov S, Evgrafov O, Ross B, et al. Mutations in LGI1 cause autosomal-dominant partial epilepsy with auditory features. *Nat Genet* 2002;30:335–41.
9. Gu W, Brodtkorb E, Steinlein OK. LGI1 is mutated in familial temporal lobe epilepsy characterized by aphasic seizures. *Ann Neurol* 2002;52:364–7.
10. Chernova OB, Somerville RPT, Cowell JK. A novel gene, LGI1, from 10q24 is rearranged and downregulated in malignant brain tumors. *Oncogene* 1998;17:2873–81.
11. Kobe B, Kajava AV. The leucine-rich repeat as a protein recognition motif. *Curr Opin Struct Biol* 2001;11:725–32.
12. Staub E, Perez-Tur J, Siebert R, et al. The novel EPTP repeat defines a superfamily of proteins with implications in epileptic disorders. *Trends Biochem Sci* 2002;27:441–4.
13. Paoli M. Protein folds propelled by diversity. *Prog Biophys Mol Biol* 2001;76:103–30.
14. Pizzuti A, Flex E, Di Bonaventura C, et al. Epilepsy with auditory features: a LGI1 gene mutation suggests a loss-of-function mechanism. *Ann Neurol* 2003;53:396–9.
15. Ottman R. Progress in the genetics of the partial epilepsies. *Epilepsia* 2001;42(suppl 5):24–30.
16. Dib C, Faure S, Fizames C, et al. Comprehensive genetic map of the human genome based on 5264 microsatellites. *Nature* 1996;380:152–4.
17. Winawer MR, Ottman R, Hauser A, et al. Autosomal dominant partial epilepsy with auditory features: defining the phenotype. *Neurology* 2000;54:2173–6.
18. Ikeda A, Kunieda T, Miyamoto S, et al. Autosomal dominant temporal lobe epilepsy in a Japanese family. *J Neurol Sci* 2000;176: 162–5.
19. Kanemoto K, Kawasaki J. Familial aphasic episodes: another variant of partial epilepsy with simple inheritance? *Epilepsia* 2000;41:1036–8.
20. Wallace RH, Izzillo P, MacIntosh AM, et al. Mutations in LGI1 in an Australian family with temporal lobe epilepsy with auditory

features. *Am J Hum Genet* 2002;71(suppl ASHG 2002 Meeting):abs 1766.

21. Bisulli F, Tinuper P, Marini C, et al. Partial epilepsy with prominent auditory symptoms: study of a family not linked to chromosome 10q. *Epileptic Disord* 2002;4:183–8.
22. Scheel H, Tomiuk S, Hofmann K. A common protein interaction domain links to recently identified epilepsy genes. *Hum Mol Genet* 2002;11:1757–62.
23. Gu W, Wevers A, Schroeder H, et al. The LGI1 gene involved in lateral temporal lobe epilepsy belongs to a new subfamily of leucine-rich repeat proteins. *FEBS Lett* 2002;519:71–6.
24. Buchanan SG, Gay NJ. Structural and functional diversity in the leucine-rich repeat family of proteins. *Prog Biophys Molec Biol* 1996;65:1–44.
25. Vicente V, Houghten R, Ruggeri Z. Identification of a site in the alpha chain of platelet glycoprotein Ib that participates in von Willebrand factor binding. *J Biol Chem* 1990;265:274–80.
26. Schneider D, Hudson K, Lin T, et al. Dominant and recessive mutations define functional domains of Toll, a transmembrane protein required for dorsal-ventral polarity in the *Drosophila* embryo. *Gene Dev* 1991;5:797–807.