

Genetic analysis of the LGI/Epitempin gene family in sporadic and familial lateral temporal lobe epilepsy.

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**Abstract.**

Mutations in the LGI1/Epitempin gene cause autosomal dominant lateral temporal lobe epilepsy (ADLTE), a partial epilepsy characterized by the presence of auditory seizures. However, not all the pedigrees with a phenotype consistent with ADLTE show mutations in LGI1/Epitempin, or evidence for linkage to the 10q24 *locus*. Other authors as well as ourselves have found an internal repeat (EPTP, pfam# PF03736) that allowed the

identification of three other genes sharing a sequence and structural similarity with LGI1/Epitempin. In this work, we present the sequencing of these genes in a set of ADLTE families without mutations in both LGI1/Epitempin and sporadic cases. No analyzed polymorphisms modified susceptibility in either the familial or sporadic forms of this partial epilepsy.

## 1. Introduction

Autosomal dominant lateral temporal epilepsy (ADLTE, OMIM#600512) is a partial epilepsy characterized by a juvenile-adult onset, rare seizures and with good response to anti-epileptic treatments, although recurrence after drug withdrawal is frequently observed. Electroencephalographic recordings of patients usually show unilateral or bilateral epileptiform activity over the temporal-occipital regions. Ottman and colleagues first described this disorder on a large pedigree that they mapped to 10q24 (Ottman *et al.*, 1995), and this linkage was later confirmed by us (Poza *et al.*, 1999). Since then, other pedigrees have been described with a clinical syndrome consistent with ADLTE, but without definite evidence of linkage to 10q24 (Mautner *et al.*, 2000; Michelucci *et al.*, 2000; Brodtkorb *et al.*, 2002). In 2002, three different groups reported mutations in LGI1/Epitempin among those families previously linked to 10q24 (Gu *et al.*, 2002; Kalachikov *et al.*, 2002; Morante-Redolat *et al.*, 2002). These were the first mutations in epilepsy to affect a non-coding gene for either an ion channel or a neurotransmitter receptor. LGI1/Epitempin is a gene with an unclear function. It was originally isolated as a tumor suppressor gene since its expression was reduced or missing in the T98G glioblastoma cell line, in which it is rearranged as a result of a t(10;19)(q24;q13) balanced

translocation (Chernova *et al.*, 1998). Krex and coworkers suggested that the functional relationship between LGI1/Epitempin and glioblastoma formation could be mediated through a different molecule that regulates its expression (Krex *et al.*, 2002). Nevertheless, Kunapuli and colleagues were unable to replicate their results, and they went further by proposing that LGI1/Epitempin not only regulates cell proliferation in T98G cells, but also invasiveness (Kunapuli *et al.*, 2003). Finally, this same group proposed that the latter action of LGI1/Epitempin was mediated by driving the expression of metalloproteinases through the ERK 1/2 pathway (Kunapuli *et al.*, 2004). Recently, Senechal *et al.* found that LGI1/Epitempin is a secreted protein, and that mutations in this gene inhibited the secretion of the protein without apparently affecting its synthesis (Senechal *et al.*, 2005).

The LGI1/Epitempin protein is characterized by the existence of two separate domains. The N-terminal region contains a putative signal peptide followed by 3 leucine-rich repeats (LRRs), which are flanked by cysteine-rich domains. On the C-terminal part of the protein, there are 7 copies of an internal repeat of 40-43 residues known as EPTP repeat (Pfam 7.2 #PF03736)(Staub *et al.*, 2002). This structural disposition defined a new gene family that includes three other members (LGI2, LGI3 and LGI4), which are mapped to 4p15.2, 8p21.3 and 19q13.12, respectively. In addition to these three genes which have a structure similar to *LGI1*, a further two genes, *VLGR1* and *TNEP1*, also contain a variable number of the EPTP repeats (Staub *et al.*, 2002).

Remarkably, most of the mutations found in LGI1/Epitempin greatly alter the primary structure of the protein (Gu *et al.*, 2002; Kalachikov *et al.*,

2002; Morante-Redolat *et al.*, 2002; Fertig *et al.*, 2003; Kobayashi *et al.*, 2003; Michelucci *et al.*, 2003; Berkovic *et al.*, 2004; Hedera *et al.*, 2004; Ottman *et al.*, 2004). Eight out of 19 published mutations provoke a truncation of the protein either by altering the reading frame of the mRNA due to the existence of insertions or deletions (5 mutations) by introducing a nonsense mutation (1 mutation), or by a single-base mutation affecting a consensus splice site (2 mutations). The remaining eleven are missense mutations found in different domains of the protein. In addition to these kindreds with mutations in LGI1/Epitempin, other pedigrees with a phenotype that is consistent with ADLTE, show no mutations in this gene nor evidence for linkage to 10q24 (Morante-Redolat *et al.*, 2002). Moreover, Bisulli and colleagues demonstrated that LGI1 does not modify the susceptibility for sporadic lateral temporal epilepsy (Bisulli *et al.*, 2004). The clinical heterogeneity observed between these pedigrees could be explained by the existence of other genetic factors underlying the disease process. In this paper, we describe the analysis of the LGI/Epitempin family of genes in those pedigrees without mutations in LGI1/Epitempin, and in a population of sporadic patients affected by lateral temporal epilepsy.

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## **2. Material & Methods.**

### 2.1. Familial cases and control populations for familial cases.

As previously described, we collected pedigrees showing the Mendelian transmission of a phenotype that is consistent with ADLTE (Morante-Redolat *et al.*, 2002; Poza *et al.* submitted). In brief, we collected families in which at least two individuals suffered from epilepsy, and if at least one of them suffered from partial auditory seizures in the absence of other neurological signs at clinical examination, and also with no known structural brain anomalies. By applying these criteria, 12 families have been identified to date (7 from Spain, 4 from Italy and 1 from Germany). No mutations in LGI1 were found in any of these families (Morante-Redolat *et al.*, 2002). In order to determine the probability that any of the variants identified in the analysis of the familial sample was pathogenic, we also collected a sample of ethnically matched neurologically normal controls consisting of 30 individuals of Basque descent (9 male, mean age at collection:  $50.29 \pm 10.27$  years), and of 30 individuals of Spanish descent (17 male, mean age at collection  $74.44 \pm 10.92$  years).

### 2.2. Sporadic cases and control population for sporadic cases.

The sporadic population was collected as described in (Bisulli *et al.*, 2004). Briefly, 38 patients (20 male, mean age at onset 19 (range 6-39)) and 41 healthy controls (19 male, mean age  $24.9 \pm 5.9$  (range 12-38)) all of Italian descent were identified, collected and analyzed as part of this study.

## Mutation screening

One case affected by each pedigree was analyzed for mutations in LGI1/Epitempin by the direct sequencing of coding exons, as previously described (Morante-Redolat *et al.*, 2002). Those without mutations underwent a further analysis for mutations in the rest of the LGI gene family members (primer sequences and PCR conditions employed for the analysis are available upon request to JP-T). All primers were designed to be able to sequence each coding exon together with at least 50 bases of the flanking introns. Primers were designed with the aid of GeneRunner (<http://www.generunner.com>) using the following Ensembl sequences LGI2: ENSG00000153012; LGI3: ENSG00000168481; LGI4: ENSG00000153902. PCR products were purified with the QIAquick PCR purification kit (Qiagen), and were subjected to cycle sequencing with fluorescently labeled dideoxynucleotides (ABIPrism BigDye v3.0, Applied Biosystems) in both senses. Sequencing products were then purified by filtration on Montage-Seq96 plates (Millipore), subjected to electrophoresis on an ABIPrism 3100 Genetic Analyzer, and were finally processed with the software provided (Applied Biosystems).

### 2.3. Analysis of polymorphisms

The population analysis of the changes identified in the sequencing of LGI1/Epitempin paralogues was performed either by RFLP when possible, or by the amplification refractory mutation system (Newton *et al.*, 1989) when a restriction digest was unsuitable. Table 1 shows the details of the assays

designed to genotype those polymorphisms in a series of healthy unrelated individuals.

All polymorphic positions are numbered according to the reference sequences from the ENSEMBL web site (<http://www.ensembl.org>); LGI2: ENST00000282970, LGI3: ENST00000306317 and LGI4: ENST00000310123

### **3. Results**

No pathogenic variant was found in any of the pedigrees. The sequencing of the LGI gene family in those families without mutations in LGI1/Epitempin allowed us to identify 8 variants (Figure 1), three of which were not in dbSNP. In an attempt to investigate whether the genetic variability found by sequencing the index case from each family was related to the disease in these families, we analyzed the segregation of each polymorphism with the disease in each of the pedigrees, and no co-segregation of the disease was found with any of the polymorphisms (Table 2). Further proof that none of the variants is related to the appearance of the epileptic phenotype in our families is seen with the presence of those same variants in a healthy population. As Table 3 shows, all the analyzed polymorphisms were also found in the general population regardless of their ethnic origin.

We then tested whether the polymorphisms identified in the *LGI* gene family could be related to an increase in the risk of the disease by using a case-control approach. We studied a population of 38 Italian sporadic patients who were previously analyzed for the presence of mutations in LGI1/Epitempin (Bisulli *et al.*, 2004), and we compared the genotypic and

allelic distributions with those obtained from a sample of 41 ethnically matched healthy controls. All genotypes were in Hardy-Weinberg equilibrium in both the case and control populations. Table 4 summarizes the result of this analysis. None of the polymorphisms showed a significant association with the disease in the LTE population.

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#### 4. Discussion.

Autosomal dominant lateral temporal epilepsy (ADLTE) is caused by mutations in *LGI1/Epitempin*, a protein that belongs to the superfamily of the leucine-rich repeat containing proteins. *LGI1/Epitempin* mutations represent a new paradigm in epilepsy research, as this epilepsy-causing gene does not encode a molecule that is directly involved in neurotransmission such as an ion channel or a neurotransmitter receptor.

Our data strongly suggest that the remaining members of the *LGI1/Epitempin* gene family do not confer an increased susceptibility with respect to ADLTE in those families without mutations in *LGI1*. These results are well in accordance with a similar study performed by Berkovic and colleagues (Berkovic *et al.*, 2004) who neither found any pathogenic mutation in any *LGI1* paralog in 2 ADLTE families nor an association between any of the polymorphisms found during the gene sequencing performed in a population of sporadic patients suffering from temporal lobe epilepsy. Interestingly enough, *LGI4* has been associated with both the familial and sporadic forms of childhood absence epilepsy (CAE) (Gu *et al.*, 2004). The lack of genetic association between the different analyzed polymorphisms and LTE in our population could be the consequence of limited statistical power to detect such an association, or alternatively, that these genes are not involved in LTE. Regarding this point, as *LGI1* has also been involved in the appearance of malignant glioblastoma, it is possible that the paralogues could also be involved in that particular phenotype or with other forms of epilepsy.

The lack of relationship between *LGI2-4* and LTE could also be explained by the fact that these genes not only exhibit an overlapping, but also a distinct expression pattern in certain cases (Senechal *et al.*, 2005). This suggests that the different members of this gene family might be related to other neurological phenotypes characterized by alterations in the same anatomical area where they are expressed. Moreover, our results do not imply that these genes might not be related to other disorders. In fact, Gu *et al.* have recently shown that this gene family is strongly conserved through evolution, which underlines the importance of its biological function, and also suggests that they may be involved in certain disorders (Gu *et al.*, 2005).

In conclusion, our analysis shows that with the exception of *LGI1* in ADLTE, the *LGI* gene family is not involved in the appearance of either familial or sporadic LTE. Further work in a larger population is needed in order to definitely rule out any genetic effect that this gene family may have on the development of this disorder. Nevertheless, the two additional genes with EPTP repeats remain as valid candidates for this disease. This could be especially true for the *VLGR1* protein as it has been shown that a mutation in one of the EPTP repeats in this protein is the cause of a natural model of audiogenic epilepsy (Skradski *et al.*, 2001).

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**Figure 1. Genomic organization of the LGI2, LGI3 and LGI4 genes.**

The positions of the polymorphisms identified in this work are shown. Gray boxes represent coding exons (drawn to scale), whereas a thin line represents introns (not drawn to scale).

**Table 1. Assays designed to study the *LGI* gene family polymorphisms.**

Gene	Localization	Change	dbSNP <sup>a</sup>	Assay <sup>b</sup>	Sense primer <sup>c</sup>	Antisense primer <sup>c</sup>	Conditions
LGI2	Intron 3	c.341+18T>C	<i>rs</i>	ARMS	TGTTTGCCACAGATCAAAA	CCGAATGTTTCAGCGAATAG (A/G)	60x30c
LGI3	Intron 4	c.422+60 A>C	<i>rs</i>	<i>BsrD I</i>	ACACGGGCTGAAATCTAG	CCATCCCAATTCTGACTC	55x35c
LGI4	Intron 2	c.242+47 A>G	rs11666576	ARMS	CCCACACATGTGCATAA (C/T)	AATGCCACCTGACATCT	F1: 60x30c <sup>e</sup> F2: 56x35c <sup>e</sup>
	Exon 5	c.456 T>C (p.H151H)	rs1673007	<i>Eco72 I</i>	CAGTGGGCCTATGTCTTT	AAGAAAAATACGTAAGAACCA	50x35c
	Exon 8	c.834 G>C (p.277P)	rs1687998	<i>EcoO109 I</i>	TTCTGCCTGTCCAGCAG	AGGTCAGAGTTTAGAGGCTT	55x35c
	Exon 8	c.960 C>G (p.A319A)	<i>rs</i>	<i>BstU I</i>	TTCTGCCTGTCCAGCAG	AGGTCAGAGTTTAGAGGCTT	55x35c
	Exon 8	c.1203 A>G (p.T400T)	rs1319969	ARMS	GCTTCGAGAGACGCAC (G/A)	TAGGTCAGAGTTTAGAGGCTT	60x30c
	Exon 9 <sup>f</sup>	c.1395 GC>AT (p.Q,L464,465Q,L)	rs12610146 rs12610234	<i>Alu I</i>	CTTCTGCTTCCAAGCTGT	CATCACCCCAAGTAGGG	55x35c

a: SNPs identified as part of this study. In *italic*, new SNPs identified in this study and submitted to dbSNP (<http://www.ncbi.nlm.nih.gov/projects/SNP/>)

b: Assay refers to the method employed for the genotyping of each polymorphism. ARMS: allele-specific PCR, rest, restriction digest performed with the indicated restriction enzyme according to the manufacture's instructions.

c: The allele-specific base is indicated in brackets. For each SNP two different reactions were performed with a common primer combined with each of the alternative primers.

d: It was necessary to use different PCR conditions for each allele-specific reaction to obtain a specific result. R1:A-allele, R2: G-allele

e: It was necessary to use different PCR conditions for each allele-specific reaction to obtain a specific result. F1:C-allele, F2: T-allele

f: Variants rs12610146 and rs12610234 always appear together in the same individual, possibly in the same allele.

**Table 2. Appearance and segregation with disease of polymorphisms in the LGI/Epitempin gene family in the familial sample.**

Family	Origin	LGI2*	LGI3*	LGI4*					
		C.341+18 T>C	c.422+60 A>C	c.242+47 A>G	c.456 T>C	c.834 G>C	c.960 C>G	c.1203 A>G	c.1395 GC>AT
ESP1	Basque	—	—	◇	—	◇	—	◇	◇
ESP2	Basque	—	—	—	—	—	—	—	—
ESP3	Basque	◇	—	—	—	—	—	—	—
ESP4	Basque	◇	—	—	—	◇	—	◇	—
ESP5	Basque	◇	—	◇	—	—	—	—	—
ESP6	Basque	◇	◇	◇	—	◇	—	◇	◇
ESP7	Basque	◇	◇	—	◇	◇	◇	◇	◇
ITA1	Italian	—	—	—	—	—	—	—	◇
ITA2	Italian	—	—	—	—	—	—	—	—
ITA3	Italian	—	—	—	—	—	—	—	◇
ITA4	Italian	◇	◇	—	—	—	—	—	—
DEU1	German	—	—	◇	—	—	—	—	◇

\* —: not present in the family; ◇: present in that family.

**Table 3. Allelic and genotypic distribution of polymorphisms found in the LGI/Epitempin family members in the familial control populations.**

Polymorphism	Control Population	Genotype frequencies* (N (%))			Allele frequencies* (%)	
		W/W	W/M	M/M	W	M
<b>LGI2</b>						
c.341+18 T>C	Basque	22 (73.3)	6 (20.0)	2 (6.6)	83.3	16.6
	Non-Basque	22 (73.3)	8 (26.7)	0 (0.0)	86.6	13.3
<b>LGI3</b>						
c.422+60A>C	Basque	20 (66.6)	9 (30.0)	1 (3.3)	81.6	18.3
	Non-Basque	18 (60.0)	10 (33.3)	2 (6.6)	76.8	23.2
<b>LGI4</b>						
c.242+47A>G	Basque	10 (33.3)	11 (36.6)	9 (30.0)	51.6	48.3
	Non-Basque	11 (36.6)	13 (43.3)	6 (20.0)	58.3	41.6
c.456T>C	Basque	27 (90.0)	3 (10.0)	0 (0.0)	95.0	5.0
	Non-Basque	25 (83.3)	5 (16.6)	0 (0.0)	91.6	8.3
c.834G>C	Basque	16 (53.3)	12 (40.0)	2 (6.6)	73.3	26.6
	Non-Basque	13 (43.3)	17 (56.6)	0 (0.0)	71.6	28.3
c.960C>G	Basque	28 (93.3)	2 (6.6)	0 (0.0)	96.6	3.3
	Non-Basque	29 (96.6)	1 (3.3)	0 (0.0)	98.3	1.7
c.1203A>G	Basque	16 (53.3)	12 (40.0)	2 (6.6)	73.3	26.6
	Non-Basque	13 (43.3)	17 (56.6)	0 (0.0)	71.6	28.3
c.1395GC>AT	Basque	17 (56.8)	11 (36.6)	2 (6.6)	75.1	24.9
	Non-Basque	19 (63.4)	9 (30.0)	2 (6.6)	78.4	21.6

\*: W refers to the wild-type allele of each polymorphism. M refers to the mutant allele at that same polymorphism.

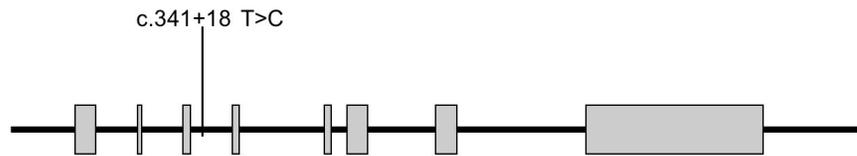
**Table 4. Allelic and genotypic distribution of polymorphisms found in the LGI/Epitempin family members in the sporadic Italian LTE cases and controls.**

Polymorphism	Control Population	Genotype frequencies <sup>a,*</sup> (N (%))				P	Allele frequencies* (%)		
		W/W	W/M	M/M			W	M	P
<b>LGI2</b>									
c.341+18 T>C	LTE cases	28 (73.3)	8 (20.0)	3 (6.6)	<i>0.733</i>	84.5	15.5	0.724	
	Controls	28 (68.8)	13 (31.7)	0 (0.0)					
<b>LGI3</b>									
c.422+60A>C	LTE cases	26 (67.6)	10 (27.0)	2 (5.4)	<i>0.489</i>	81.1	18.9	0.861	
	Controls	25 (61.0)	16 (39.0)	0 (0.0)					
<b>LGI4</b>									
c.242+47A>G	LTE cases	14 (36.6)	14 (36.6)	10 (26.8)	0.725	54.9	45.1	0.678	
	Controls	15 (36.6)	18 (43.9)	8 (19.5)					
c.456T>C	LTE cases	30 (80.0)	6 (16.6)	1 (3.3)	0.993	88.3	11.6	0.864	
	Controls	33 (80.5)	7 (17.1)	1 (2.4)					
c.834G>C	LTE cases	22 (56.6)	16 (43.2)	1 (2.7)	<i>0.978</i>	76.6	23.3	0.795	
	Controls	23 (56.1)	18 (43.9)	0 (0.0)					
c.960C>G	LTE cases	37 (97.3)	1 (2.7)	0 (0.0)	<i>0.663</i>	98.6	1.3	0.349	
	Controls	38 (92.7)	3 (7.3)	0 (0.0)					
c.1203A>G	LTE cases	22 (56.6)	15 (40.0)	1 (3.3)	<i>0.705</i>	76.6	23.3	0.934	
	Controls	22 (53.7)	19 (46.3)	0 (0.0)					
c.1395GC>AT	LTE cases	22 (56.6)	13 (33.3)	4 (10.0)	0.287	73.3	26.7	0.166	
	Controls	16 (39.0)	20 (48.8)	5 (12.2)					

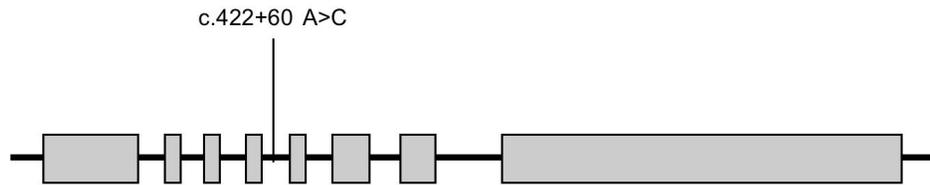
a: For those values in *italics*, the chi-square test has 1df either because of the low number or the complete absence of the individuals' homozygous genotype for the rare allele. The analysis was performed by grouping heterozygous and homozygous genotypes for the rare allele.

\*: W refers to the wild-type allele of each polymorphism. M refers to the mutant allele at that same polymorphism.

***LGI2***



***LGI3***



***LGI4***

