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Analysis of LGI1 promoter sequence, PDYN and GABBR1 polymorphisms in sporadic and familial lateral temporal lobe epilepsy

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Abstract

Autosomal dominant lateral temporal epilepsy (ADTLE) is a genetically transmitted epileptic syndrome characterized by focal seizures with predominant auditory symptoms likely originating from the lateral region of the temporal lobe. Mutations in coding region or exon splice sites of the leucine-rich, glioma-inactivated 1 (LGI1) gene account for about 50% of ADLTE families. *De novo* LGI1 mutations of the same kind have also been found in about 2.5% of non-familial cases with idiopathic partial epilepsy with auditory features (IPEAF). In both conditions, mutations in the LGI1 promoter region have not been reported. We sequenced the minimal promoter region of LGI1 in the probands of 16 ADLTE families and in 104 sporadic IPEAF patients and no mutations clearly linked to the disease were found. However, two polymorphisms, −500G > A and −507G > A, with potential functional implications were identified and analysed in the cohort of sporadic IPEAF patients but their frequencies did not differ from those found in a control population of similar age, gender and geographic origin. We also analysed in our study population the

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GABA(B) receptor 1 c.1465G > A and the prodynorphin promoter 68-bp repeat polymorphisms, previously associated with temporal lobe epilepsy. None of these polymorphisms showed a significant association with IPEAF, whereas a tendency towards association with the prodynorphin low expression (L) alleles was found in the small group of ADLTE index cases, in agreement with previous studies suggesting that this polymorphism is a susceptibility factor in familial forms of temporal lobe epilepsy.

Keywords: Lateral temporal epilepsy; LGI1 promoter; Prodynorphin gene; GABBR1; Association study

Autosomal dominant lateral temporal epilepsy (ADTLE; OMIM 600512), also named autosomal dominant partial epilepsy with auditory features (ADPEAF), is a genetically determined form of temporal lobe epilepsy (TLE) characterized by typical auditory auras and/or other less frequent symptoms, such as aphasic or visual auras, suggesting a lateral temporal onset [10,15,24]. Other features of this syndrome include absence of brain abnormalities detectable by standard magnetic resonance imaging (MRI), and good outcome. The leucine-rich, glioma inactivated 1 (LGI1) gene, which is expressed mainly in neurons, has been shown to harbour mutations responsible for ADLTE in its coding region or at splice sites [8,13]. Overall, LGI1 mutations account for about 50% of ADLTE families [1,10,16].

Sporadic (non-familial) cases with idiopathic partial epilepsy with auditory features (IPEAF) have been described [2]. Similarly to the majority of ADLTE cases, these patients have auditory partial epilepsy, normal MRI, and benign outcome, but they have no family history of epilepsy. Sequence analysis of LGI1 exons in IPEAF patients revealed two *de novo* LGI1 mutations [3,11], providing a link between familial and sporadic patients with auditory partial epilepsy.

The LGI1 gene is variably expressed across the human and mouse brain tissues [5,8,18]. The regulation of LGI1 expression is still largely unknown. The minimal promoter sequence necessary for LGI1 transcription *in vitro* has been shown to lie in the 531 bp immediately upstream of exon 1 [19]. Analysis of this regulatory region in epilepsy patients has not been reported.

Genetic polymorphisms with an impact on expression or function of genes potentially implicated in epilepsy have been found associated with TLE. The prodynorphin (PDYN) gene encodes several peptides, dynorphins, which exert an anticonvulsant role in the brain. Therefore, this gene is regarded as a potential susceptibility gene for epilepsy. Its expression is modulated by a 68-bp variable number of tandem repeat (VNTR) polymorphism occurring in its core promoter region, the number of repeats determining either low (L) or high (H) expression levels of this gene [25]. Recently, Stogmann et al. [20] reported an association between TLE and PDYN low-expression (L) alleles in patients with family history of epilepsy. Subsequent attempts to replicate this finding gave negative [6,23] or inconclusive [4] results, making it unclear whether PDYN has an important effect on the development of temporal lobe epilepsy.

The GABA(B) receptor 1 is one of the two receptors for the gamma-aminobutyric acid (GABA), the main inhibitory neurotransmitter in the central nervous system. The gene encoding this receptor, GABBR1, exhibits a polymorphic nucleotide change, c.1465G > A, allowing a Gly489Ser substitution, which likely affects its function. This polymorphism was initially found to be associated with TLE in a cohort of patients from South Italy [7], but this finding has not been replicated in several case-control studies performed on groups of patients from other countries [9,17,21,22].

In this paper we report the analysis of the LGI1 promoter sequence and of the PDYN and GABBR1 polymorphisms in a series of 120 Italian patients with familial or sporadic lateral temporal lobe epilepsy.

We studied a total of 120 Italian epileptic patients. Sixteen of these were probands of ADLTE families, each family having at least two members suffering from lateral temporal epilepsy [10; Diani et al., submitted for publication]. One hundred and four sporadic IPEAF cases (55 men, 49 women; average age at onset 19.2 (range 4–41) years) were selected on the basis of the auditory features of their auras and absence of first- and second-degree relatives with epileptic seizures [2]. We also collected a sample of neurologically normal Italian controls consisting of 103 unrelated individuals of Italian descent (45 men, 58 women).

Mutation analysis of the minimal LGI1 promoter region from −531 to +84 [19] (position +1 corresponding to the first nucleotide of the NCBI sequence no. AF055636) was performed in our patients by direct sequencing. PCR amplification of this sequence was carried out at 61 °C (30 cycles) in a PCR machine (MWG) using the primers: left, cacttctgtttcccttccttgtc; right, agctgattcgtgagcttctcct. Sequencing of PCR products was performed using the Big Dye Terminator Cycle Sequencing kit (ABI PRISM, Applied Biosystems) and an ABI3730 automatic sequencer (Applied Biosystems). The promoter region around polymorphisms −500G > A and −507G > A was analysed in healthy controls by denaturing high-performance liquid chromatography (Transgenomic; conditions available on request). Search for transcription factor binding sites in the LGI1 promoter sequence was carried out by using the transcription element search software (TESS) available at www.cbil.upenn.edu/tess/.

PDYN promoter genotypes were determined by PCR as described in Stogmann et al. [20]; alleles 1 and 2 were grouped as low-expression (L) allele, alleles 3 and 4 as high-expression (H) allele. The GABBR1 polymorphism c.1465G > A was analysed in cases and controls as described previously [7]. Statistical significance of allelic and genotypic contingency tables was assessed using the chi-square distribution.

We sequenced the LGI1 promoter region in the probands of 16 typical ADLTE families (including four families with LGI1 mutations) and in 104 sporadic IPEAF patients. No nucleotide changes either co-segregating with the disease in families or appearing de novo in sporadic cases were detected. This analysis, however, revealed two single nucleotide polymorphisms

Table 1
Allelic and genotypic distribution of LGI1 and PDYN promoter polymorphisms in the IPEAF and control populations

Polymorphism	Population	Genotype frequencies[a] (*N* (%))			Allele frequencies[a] (*N* (%))		Genotype relative risk[b]
		W/W	W/M	M/M	W	M	
LGI1	Sporadic cases	95 (91.3)	9 (8.7)	0 (0.0)	199 (95.6)	9 (4.4)	3.57
−500G > A	Controls	97 (94.1)	6 (5.9)	0 (0.0)	200 (97.1)	6 (2.9)	
LGI1	Sporadic cases	101 (97.1)	3 (2.9)	0 (0.0)	205 (98.5)	3 (1.5)	4.30
−507G > A	Controls	99 (96.1)	4 (3.9)	0 (0.0)	202 (98.1)	4 (1.9)	
PDYN	Sporadic cases	55 (52.9)	41 (39.4)	8 (7.7)	151 (72.6)	57 (27.4)	1.99
repeat	Controls	51 (49.5)	43 (42.8)	9 (8.7)	145 (70.4)	61 (29.6)	

[a] W refers to the wild-type allele of each polymorphism; M refers to the mutant allele at that same polymorphism; *p*-values were not significant.
[b] Estimates of the detectable genotype relative risk for a power of 80% at a significance level of 0.05. Calculations were done using the CaTS program available at http://www.sph.umich.edu/csg/abecasis/CaTS/index.html.

(SNPs), one of which, −507G > A, was already present in public data bases (rs3758532), whereas the other, −500G > A, was not. These SNPs are localized at the border of a stretch of sequence conserved in the mouse *Lgi1* promoter (Fig. 1). Analysis of the genomic sequence around these two polymorphisms using the TESS program (www.cbil.upenn.edu/tess) revealed that the −500G > A polymorphism is localized within a predicted AP-2 transcription factor binding site, whereas the −507G > A variant lies only two base pairs upstream of the same site (Fig. 1). AP-2 is one of the most critical transcription factors in neural gene expression [12]. Therefore, these SNPs might be functionally important. Analysis of their allelic and genotypic frequencies in our population of sporadic IPEAF patients and in a series of 103 healthy controls of similar age, gender, and geographic origin showed no significant differences (Table 1). All studied genotype distributions were in good agreement with the Hardy–Weinberg equilibrium.

We also tested allelic and genotypic frequencies of the PDYN 68-bp VNTR polymorphism in the same population of epileptic patients. No significant differences were observed between the groups of sporadic patients and controls (Table 1). Instead, in the small group of ADLTE probands, an increase of L-alleles ($p = 0.022$) and of L/L and L/H genotypes ($p = 0.061$) was observed in comparison with the control population (Table 2). These results may suggest a trend toward association between PDYN and ADLTE, to be confirmed in a larger study population.

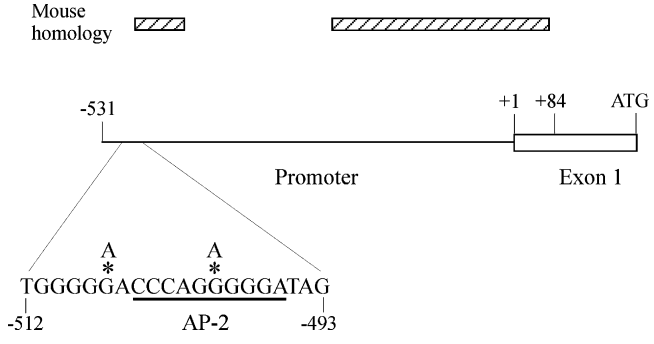

Fig. 1. Schematic representation of the minimal promoter region of LGI1. Empty box, untranslated portion of exon 1; thin line, promoter region upstream of exon 1; dashed box, homology region with mouse *Lgi1* promoter. The 20-bp sequence around the polymorphisms −500G > A and −507G > A is shown; the 10-bp putative AP-2 transcription factor recognition sequence is underlined.

Finally, the GABBR1 c.1465G > A minor allele was absent in both familial and sporadic cases, and it was found only in one control subject, in agreement with its very low frequency in the Italian population (about 0.5%) [7].

Mutations in the coding sequence or exon splice sites of LGI1 are found in about 50% of families with ADLTE and in about 2.5% of sporadic IPEAF cases [2,3,8,10,11,13,16]. Nucleotide changes in the LGI1 promoter sequence that influence gene expression may also modify susceptibility to ADLTE or IPEAF. Analysis of the LGI1 promoter sequence revealed no mutations segregating with ADLTE in our families without mutations in the LGI1 coding sequence. In addition, two promoter polymorphisms of potential functional significance showed no allelic and genotypic differences between the IPEAF and control populations. This negative result, however, does not exclude the possibility that these polymorphisms may exert a minor effect on susceptibility to IPEAF, perhaps detectable in a larger population of patients, or that they are involved in other forms of epilepsy as modifying factors.

Stogmann et al. [20] reported an association between PDYN promoter L-alleles and TLE in a cohort of 43 patients of middle-European descent whose first- and/or second-degree relatives also had epilepsy (any syndrome) or febrile seizures (FS). In these patients epilepsy was often severe or preceded by FS. Subsequent association studies of familial-risk TLE patient populations failed to replicate this finding [6,23] or gave inconclusive results [4]. Taken individually, these failures might in part

Table 2
Distribution of allelic and genotypic frequencies of the PDYN promoter polymorphism in ADLTE index cases and controls

	Controls (*N* (%))	ADLTE cases (*N* (%))
Alleles (*n*)	206	32
L	61 (29.6%)	16 (50.0%)
H	145 (70.4%)	16 (50.0%)
p-Value	–	0.022[a]
Genotypes (*n*)	103	16
L/L	9 (8.7%)	3 (18.8%)
L/H	43 (41.8%)	10 (62.4%)
H/H	51 (49.5%)	3 (18.8%)
p-value	–	0.061[b]

[a] *p*-Value from χ^2-test using 1 degree of freedom.
[b] *p*-value from χ^2-test using 2 degrees of freedom.

be ascribed to the small sizes of study populations. However, a recent stratified Mantel-Haenszel analysis of all previous works found a cumulative significant association between familial-risk TLE cases and PDYN promoter L alleles [14]. In our study population there was a trend towards association with the PDYN promoter L-alleles in the group of probands of ADLTE families but not in the cohort of sporadic IPEAF cases, further supporting a role of the prodinorphyn gene as a modifier of susceptibility to familial temporal lobe epilepsy.

Finally, our study showed that there is no association between the GABBR1 c.1465G > A polymorphism and lateral temporal lobe epilepsy.

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