Association of intronic variants of the *KCNAB1* gene with lateral temporal epilepsy

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Summary The *KCNAB1* gene is a candidate susceptibility factor for lateral temporal epilepsy (LTE) because of its functional interaction with *LGI1*, the gene responsible for the autosomal dominant form of LTE. We investigated association between polymorphic variants across the *KCNAB1* gene and LTE. The allele and genotype frequencies of 14 *KCNAB1* intronic SNPs were determined in 142 Italian LTE patients and 104 healthy controls and statistically evaluated. Single SNP analysis revealed one SNP (rs992353) located near the 3′end of *KCNAB1* slightly associated with LTE after multiple testing correction (odds ratio = 2.25; 95% confidence interval 1.26—4.04; $P = 0.0058$). Haplotype analysis revealed two haplotypes with frequencies higher in cases than in controls, and these differences were statistically significant after permutation tests (Psim = 0.047 and 0.034). One of these haplotypes was shown to confer a high risk for the syndrome (odds ratio = 12.24; 95% confidence interval 1.32—113.05) by logistic regression analysis. These results support *KCNAB1* as a susceptibility gene for LTE, in agreement with previous studies showing that this gene may alter susceptibility to focal epilepsy.

Introduction

Lateral temporal epilepsy (LTE) comprises a minority (about 10%) of all temporal epilepsies (Williamson and Engel, 2008). It is currently distinguished from mesial temporal epilepsy, owing to its specific seizure characteristics and low proportion of lesional cases (Florindo et al., 2006). Families and sporadic cases with non-lesional LTE have been reported in the past years, the former under the terms autosomal dominant partial epilepsy with auditory features (ADPEAF; Ottman et al., 1995) or autosomal dominant lateral temporal epilepsy (ADLTE; Poza et al., 1999), the latter as idiopathic partial epilepsy with auditory features (IPEAF; Bisulli et al., 2004a). Clinically, both conditions are characterized by focal seizures with auditory auras, high propensity to generalize, negative MRI findings, and relatively benign evolution (Michelucci et al., 2003; Ottman et al., 2004).

Mutations in the leucine rich, glioma inactivated 1 (*LGI1* gene) have been found in about 50% of ADLTE families (Kalachikov et al., 2002; Morante-Redolat et al., 2002; Michelucci et al., 2003; Ottman et al., 2004). Over 25 *LGI1* mutations have been reported thus far (Nobile et al., 2009), nearly all of which have been unique to an individual family. *De novo LGI1* mutations have been found in two sporadic LTE patients out of 104 sporadic cases analysed (Bisulli et al., 2004b; Michelucci et al., 2007).

The *LGI1* gene is mainly expressed in neurons and its function is still unclear. Its protein product, Lgi1, contains two distinct domains, a N-terminal LRR domain (Kobe and Kajava, 2001), and a C-terminal EPTP (beta-propeller) domain (Staub et al., 2002), each mediating protein-protein interactions. The Lgi1 protein has recently been shown to form a complex with the rapidly inactivating Kv1 potassium channel (Schulte et al., 2006). This channel is located mainly at presynapses and consists of two pore-forming alpha subunits, Kv1.1 and Kv1.4, and one beta subunit, Kvbeta1. In response to depolarisation, Kvbeta1 occludes the channel pore, thus inactivating the channel. In vitro experiments have shown that Lgi1 selectively antagonizes the function of Kvbeta1, thereby preventing inactivation of Kv1 channels (Schulte et al., 2006). These results suggest that *LGI1* mutations may cause changes in inactivation gating of neuronal Kv1 channels, thereby giving rise to epileptic activity. Accordingly, the gene encoding Kvbeta1, *KCNAB1*, can be regarded as a candidate susceptibility gene for lateral temporal epilepsy. Analysis of 9 *LGI1*-negative families with typical ADLTE, however, failed to identify any mutations in this gene and in those coding for Kv1.1 and Kv1.4 (Diani et al., 2008). This negative result suggests that *KCNAB1* does not act as a major gene in ADLTE, leaving the possibility that it may contribute to susceptibility to non-mendelian forms of LTE.

A recent association study of 2700 epilepsy patients, belonging to four different countries (UK, Ireland, Finland, and Australia) and exhibiting various kinds of syndromes and seizure types (either generalized or partial), identified a number of SNPs located within several genes that contributed to susceptibility to epilepsy in specific populations. The most significant associations were found between two common *KCNAB1* intronic variants and a Finnish population of 489 focal epilepsy patients (Cavalleri et al., 2007). *KCNAB1* common polymorphisms may therefore also influence the risk of focal epilepsy in other geographical populations and/or in particular types of focal epilepsy. In particular, because of its possible functional association with the *LGI1* gene, *KCNAB1* might be a susceptibility gene for lateral temporal epilepsy. To test this hypothesis, we analysed 14 non-coding variants scattered over the *KCNAB1* gene in a series of Italian patients with sporadic LTE and in geographically matched controls.

Patients and methods

Study subjects

The study group consisted of 142 unrelated patients (68 women and 74 men) identified either prospectively or retrospectively in numerous Italian epilepsy centres from 2002. Diagnosis of LTE was based mainly on a clinical history of at least two lifetime seizures with auditory features and absence of clear-cut neuroradiological abnormalities including mesial temporal sclerosis, as reported previously (Bisulli et al., 2004a). All affected individuals were interviewed and directly examined by expert clinical epileptologists (R.M., F.B., P.T., P.S., S.S., A.T.G., C.D.B., E.F., T.G., G.C., A.B., U.A., V.C., M.E., G.G., S.C., M.M., S.B., E.F., A.P., P.V., C.B.). The mean age at seizure onset was 19.3 (range 4—63). Neurological examination results were normal in all patients and response to therapy was good in most patients (Bisulli et al., 2004a). Interictal EEG recordings showed epileptiform abnormalities only in a minority of cases. Of the 142

patients, 129 (90.8%) were sporadic (non-familial) cases, whereas 13 (9.2%) had a family history of seizures or febrile convulsions in one or more relatives but did not belong to ADLTE families. Index cases from ADLTE families, here defined as families with 3 or more epileptic patients over 2 or more generations with at least 2 patients concordant for ictal auditory symptoms, were excluded from analysis. Cases with auditory symptoms accompanying but not preceding, at least in part of the seizures reported, other temporal lobe symptoms, or lacking neuroimaging data were also excluded. All patients analysed had no mutations in LGI1. The healthy control group comprised 104 subjects without history of epilepsy or other neurological conditions. They were recruited in the same geographical regions of Italy as the patients, in order to minimize population stratification effects. Blood samples were drawn from each individual after obtaining informed consent and genomic DNA was extracted by using a standard protocol.

The relatively small size of the study group is justified by its very narrow clinical definition. We only included patients with auditory focal seizures of probable lateral temporal origin, clinically indistinguishable from the majority of patients with ADLTE. We did so on the assumption that susceptibility to LTE is more likely influenced by variants in genes that are functionally linked to *LGI1* than in other types of genes.

SNP typing

Fourteen intronic SNPs were chosen within the *KCNAB1* gene according to the following criteria: high minor allele frequency (MAF; >0.324), closeness to an exon or group of exons, and occurrence at a restriction enzyme sequence made (un)cleavable by the genetic variation. Six of them are HapMap TagSNPs (rs9816126, rs98766870, rs1546750, rs2720281, rs2280299 and rs2280032). A few SNPs were tested by allele specific oligonucleotide (ASO) PCR (conditions available on request). The following SNPs were chosen (corresponding restriction site or ASO in parentheses): rs1386956 (ScaI), rs9816126 (DraI), rs728382 (TaqI), rs4679773 (ASO), rs9876870 (TaqI), rs3755631 (ASO), rs2280561 (NlaIII), rs1546750 (ApaLI), rs17352408 (PstI), rs2720281 (MspI), rs4295133 (MspI), rs2280299 (DraI), rs2280032 (StuI), and rs992353 (NdeI). PCR primers (sequences available on request) were designed using the Primer 3 program (http://www.bioinformatics.nl). Following PCR reactions, amplified DNA fragments were digested by the corresponding restriction enzyme (conditions available on request), resolved on a 2% agarose gel, and visualized by ethidium bromide straining.

Statistical analysis

Genotype and allele frequencies, and Hardy—Weinberg equilibrium tests of the *KCNAB1* SNPs were performed separately among cases and controls using the SNPStats (Solé et al., 2006) and HaploView (http://www.broad.mit.edu/mpg/haploview/) programs.

Estimation of power to detect association between *KCNAB1* SNPs and LTE was evaluated using the Power of Genetic Analysis (PGA) package which comprises algorithms and graphical user interfaces for minimum detectable risk

calculations using SNP or haplotype effects under different study constrains (Menashe et al., 2008). The minimum detectable effects with odds ratio (ORs) was calculated at various allele frequencies of SNP allele in general (co-dominant) model, based on an alpha of 0.001 and prevalence 1/10.000 in the general population. With this sample size, we have 80% power to detect an OR of 2.5 if the SNP allele frequency is 40% for single SNP analysis, while risk-influencing haplotype analysis detects an OR of 2.2 under the same study constrains.

The χ^2 and *P*-value for the allele frequencies in cases vs. controls were calculated for each polymorphism with the HaploView program. Moreover, unconditional logistic regression analysis was performed with SNPStats, to investigate association between SNP genotype distribution and epilepsy status in the sample.

Because the mode of inheritance was unknown, three genetic models (co-dominant, dominant and recessive) were considered and ORs and 95% confidence intervals (CIs) were calculated. This analysis is slightly more powerful when the significance threshold is properly determined (Lettre et al., 2007). The homozygous genotype for the wild type allele was considered the referent class (set as having risk = 1) and *P* values were derived from a likelihood ratio test (LRT). Linkage disequilibrium (LD) between the SNPs was tested using SNPStats and HaploView software version 4.1. To account for multiple testing, we used the Single Nucleotide Polymorphism Spectral Decomposition (SNPSpD) program to correct the significance threshold taking into account LD between SNPs (http://gump.qimr.edu.au/general/daleN/SNPSpD).

Based on genotypic information of the 14 *KCNAB1* SNPs, an LD block map was constructed with the HaploView software. The block structure was based on measures of *D'* and the haplotype block definition was performed using *D'* with a threshold of 0.85 (Lewontin, 1988). Haplotype association analysis was performed with HaploView and SNPStats. Haplotype phase and population frequency were inferred using a standard expectation-maximization algorithm (EM) from both programs. Distribution of haplotypes was compared in the epileptic and control subjects and results were obtained with a simple χ^2 tests from HaploView. Permutation tests were used to control multiple testing errors with 100,000 simulations. Regression-based haplotype association test through an LTR was performed and ORs and 95% CIs were generated for each haplotype and compared to the most common haplotype with the SNPStats program. Haplotypes with frequencies greater than 1.5% were considered.

Results

SNP analysis

The distribution of the 14 SNPs with respect to *KCNAB1* exons is shown in Fig. 1. They were chosen based on their high MAF and closeness to single exon or exon clusters. Most SNPs altered/created a restriction site and were typed by restriction fragment length polymorphism (RFLP) analysis, the remaining by ASO PCR (see Methods: SNP typing).

We analysed these SNPs in a cohort of 142 Italian LTE cases and in a matching control group of 104 healthy subjects from the same geographical areas of Italy. Their allele fre-
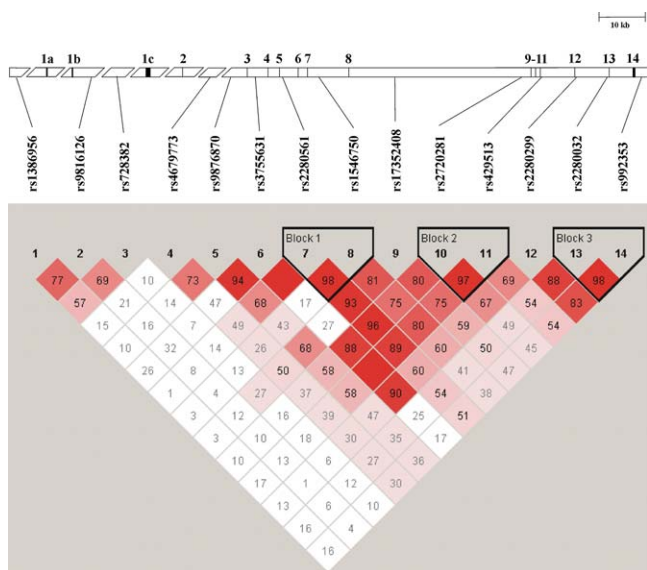
Figure 1 SNP distribution along the *KCNAB1* gene. (Top) The *KCNAB1* exons are shown as boxed vertical lines and numbered. The map position of the SNPs analysed with respect to exons is indicated. (Bottom) Description and linkage disequilibrium pattern with block structure (*D'*) of the *KCNAB1* gene region. SNPs are consecutively numbered 1—14. Blocks of strong linkage disequilibrium between neighboring SNPs are indicated.

quencies were similar to those reported in the NCBI database for the Caucasian population. The genotype distributions of the SNPs evaluated in this study were in Hardy—Weinberg equilibrium in both groups (data not shown).

Comparisons of allelic frequencies did not reveal any statistically significant association between LTE cases and controls (data not shown). Yet, by comparing the overall genotype frequency distribution, we found statistically significant differences for the SNPs rs992353 and rs2280032 (*P* values 0.0058 and 0.028, respectively; Table 1). Of these SNPs, only rs992353 remained slightly associated (dominant model) with LTE after correction for multiple testing made using the program SNPSpD, which set the significance threshold for our study population at $P = 0.006$. Thus, under a dominant model assumption, heterozygotes and homozygotes for the rs992353 minor allele (GA or GG) confer a higher risk of LTE in comparison with homozygotes for the common allele (AA) (OR = 2.25; 95% CI 1.26—4.01; Table 1).

Linkage disequilibrium and haplotype analyses

We constructed an LD block map of the *KCNAB1* gene region in our study population. *D'* values calculated with the HaploView software revealed three independent blocks of LD each made of two SNPs: the first block (rs2280561 and rs1546750) spans exons 6 and 7, the second (rs2720281 and rs429513) exons 9, 10 and 11, and the third (rs2280032 and rs992353) exons 13 and 14 (Fig. 1). This LD block map was similar to that constructed using the HapMap Project data (not shown). We also conducted a haplotype association analysis using all SNPs in order to investigate their combined effect on epilepsy risk. Haplotypes were inferred by expectation-maximization algorithm implemented in HaploView and SNPStats softwares and 15 haplotypes that had frequency >0.015 were included in the analysis, as shown in Table 2. The most common haplotype GGACTCCTAAACGA containing no risk allele at each SNP locus had similar frequencies in controls and cases, 16.6% and 17.2%, respectively (*P* = 0.86, Psim = 1), as shown in score test of haplotype distribution. Two haplotypes, GGGGCCTGGGGGTTG and GGGCTCTGGGGGTTG, had higher frequencies in cases than in controls and these

Table 1 Genotype frequencies of KCNAB1 among cases and controls, and risk of epilepsy (n = 246).

SNP	Model	Genotype	Controls n (%)	Cases n (%)	P^a	Psim[b]	OR (95% CI)	P^c
							Logistic regression	
rs2280032	Codominant	GG	40 (38.5%)	36 (25.4%)	0.198	0.844	1	0.05
		TG	42 (40.4%)	78 (54.9%)			2.06 (1.15—3.71)	
		TT	22 (21.1%)	28 (19.7%)			1.41 (0.69—2.90)	
	Dominant	GG	40 (38.5%)	36 (25.4%)			1	0.028
		TG + TT	64 (61.5%)	106 (74.7%)			1.84 (1.07—3.18)	
	Recessive	GG + TG	82 (78.8%)	114 (80.3%)			1	0.78
		TT	22 (21.1%)	28 (19.7%)			0.92 (0.49—1.71)	
rs992353	Codominant	AA	36 (34.6%)	27 (19.0%)	0.088	0.525	1	0.015
		GA	46 (44.2%)	85 (59.9%)			2.46 (1.33—4.56)	
		GG	22 (21.1%)	32 (21.1%)			1.82 (0.87—3.82)	
	Dominant	AA	36 (34.6%)	27 (19.0%)			1	0.0058*
		GA + GG	68 (65.4%)	115 (81%)			2.25 (1.26—4.04)	
	Recessive	AA + GA	82 (78.8%)	112 (78.9%)			1	1
		GG	22 (21.1%)	30 (21.1%)			1.00 (0.54—1.86)	

[a] Chi-square test *P*-value.
[b] 100,000 Permutation Multiple tests correction.
[c] Likelihood ratio *P*-value.
* Statistically significant after multiple testing correction.

Table 2 Frequency (%) and associations between haplotypes of KCNAB1 and risk of epilepsy.

Haplotype	Control %	Case %	P^a	$Psim^b$	OR (95% CI)^c	P^d
GGACTCCTAAACGA	16.6	17.2	0.861	1	1(reference)	—
ATGGCCTGGGGTTG	8.2	2.8	0.007	0.002	0.38 (0.14—1.07)	0.067
GGGGCCTGGGGTTG	1.6	5.9	0.017	0.034	12.24 (1.32—113.05)	0.028
GGGCTCTGGGGTTG	0.1	2.3	0.037	0.047	—	<0.0001
GGACCCTGGGGTTG	0.5	2	0.158	0.845	2.02 (0.32—12.87)	0.46
ATACTCCTAAACGA	1.8	0.5	0.181	0.867	0.40 (0.04—4.08)	0.44
GGGCTCCTAAACGA	3.5	1.6	0.168	0.865	0.48 (0.09—2.43)	0.38
GGAGCCTGAAGTTG	3.4	1.6	0.202	0.881	0.75 (0.18—3.08)	0.69
GGAGCCTGGGGTTG	4.8	3.1	0.318	1	0.88 (0.29—2.60)	0.81
ATGCTCCTAAACGA	4.5	3	0.387	1	0.87 (0.22—3.48)	0.85
ATGGCCTGGGGCGA	2.3	2.6	0.832	1	3.88 (0.60—25.24)	0.16
GGACTCCTAAATTG	2.5	1.9	0.640	1	1.11 (0.23—5.27)	0.9
GGACTCCTAAATGA	2.1	1.9	0.836	1	1.25 (0.18—8.96)	0.82
GGACTCTGAGGTTG	1.6	2.1	0.685	1	1.10 (0.28—4.41)	0.89
GGACTCTGGGGTTG	1.7	1.2	0.639	1	1.69 (0.22—12.99)	0.62

[a] P values derived by simple χ^2 tests.
[b] Multiple test correction generated by 100,000 simulations.
[c] Association derived by likelihood ratio test (LRT).
[d] Likelihood ratio P-value.

differences were statistically significant after permutation tests (Psim = 0.034 and 0.047, respectively; Table 2). Logistic regression analysis performed with SNPStats also showed association of both haplotypes (P = 0.028 for GGGGCCTGGGGTTG; P = 0.0001 for GGGCTCTGGGGTTG). In addition, the GGGGCCTGGGGTTG haplotype was found to confer a high risk for LTE (OR = 12.24; 95% CI 1.32—113.05), whereas the GGGCTCTGGGGTTG haplotype was estimated at too low frequency in controls to obtain an OR score by regression analysis.

Notably, the ATGGCCTGGGGTTG haplotype was more frequent in controls (8.2%) than in cases (2.8%) and this frequency difference was statistically significant after permutation tests correction (Psim = 0.002). Yet, using the regression model, the negative association with the haplotype ATGGCCTGGGGTTG showed only marginal significance (P = 0.067; Table 2).

Discussion

Genetic predisposition to LTE may be influenced by the *KCNAB1* gene, which is functionally related to *LGI1*. The results presented in this paper support *KCNAB1* as a susceptibility gene for LTE.

Earlier, two *KCNAB1* SNPs (rs3755631 and rs16826199, which are very close to each other (1500 base pairs) and in strong LD) were found associated with partial epilepsy in a Finnish group of 489 patients and the most stringent P value was obtained in a subgroup of 371 subjects with secondarily generalized tonic—clonic seizures (Cavalleri et al., 2007). In the same work, this finding was not replicated in cohorts of comparable sizes of partial epilepsy patients from UK and Ireland, suggesting that the effect of these polymorphisms was population-specific (Cavalleri et al., 2007).

To test whether these variants might also confer risk for LTE, which is characterized by frequent secondary general-

ization of seizures (Bisulli et al., 2004a), we analysed SNP rs3755631, which is located near exon 3, in our groups of Italian cases and controls but failed to detect any association. This failure could be explained by either a lack of effect of rs3755631 on LTE or a population-specific effect. Considering the genetic distance of the Finnish population from the Italian (and Irish and UK) populations, the latter seems to be a more reliable explanation: if *KCNAB1* has any influence on risk for LTE, then different susceptibility variants may be found in different geographic populations. Indeed, analysis of 13 additional SNPs across the gene in our cohort showed association with rs992353, located near exon 14, with a P value slightly below the significance threshold after correction for multiple testing, suggesting an association trend of this variant with LTE. This trend was confirmed by haplotype analysis of all the 14 SNPs, which showed association of two *KCNAB1* haplotypes with LTE, still significant after permutation test correction (100,000 tests). In addition, logistic regression analysis showed that haplotype GGGGC-CTGGGGTTG conferred a high risk for LTE (OR = 12.24; 95% CI 1.32—113.05). Although the significance level was borderline in both single SNP and haplotype analyses, these results, taken together, support the existence of variants of moderate to strong effect that confer risk for LTE in the Italian population.

Identifying a phenotype is the key to most genetic studies, and strict or narrow inclusion or exclusion criteria can reduce heterogeneity (Rees, 2007). The epileptic phenotype analysed in this paper represents a well defined, particular subtype of focal epilepsy in which seizures likely originate from the lateral temporal cortex. Hence, it is reasonable to hypothesize that LTE is genetically more homogeneous than the broad class of focal epilepsies and that a relatively limited number of genetic variants confer risk for LTE. Within the limits of these assumptions, our findings support the notion that *KCNAB1* confers susceptibility to LTE. Other particular forms of focal epilepsies may also be influenced by

KCNAB1 variants. Hints for this notion were already provided by the stratified association analysis of the Finnish cohorts in Cavalleri and co-workers (2007), in which the top *P* value obtained in the subgroup of patients with secondarily generalized tonic—clonic seizures was about 1 order of magnitude below that of all focal epilepsies and 2 orders below that of other test phenotypes. Owing to the different phenotypes examined, our study does not replicate that by Cavalleri and co-workers. However, our results suggest that, following initial studies of large sets of patients with inevitably heterogeneous phenotypes, analysis of well defined subtypes of epilepsy that, presumably, are genetically more homogeneous may help dissect the susceptibility components of a given predisposing gene.

Some limitations of our study have to be taken into account: our sample size is not large enough to detect gene variations with low-moderate effect on phenotype; in addition, by analysing a single gene we were not able to evaluate the epistatic effect of more genes on total phenotypic variance; and finally, we did not include factors in our regression model that could confound SNP association with LTE. Therefore, more studies are needed to confirm the role of *KCNAB1* variants in susceptibility to LTE and possibly other focal epilepsies in different geographic populations.

Conflict of interest

The authors have reported no conflicts of interest.

Acknowledgments

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