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SHORT COMMUNICATION

Failure to confirm association of a polymorphism in KCNMB4 gene with mesial temporal lobe epilepsy

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Summary A recent study has implicated a tagging single nucleotide polymorphism (SNP) rs398702 located 3′ of KCNMB4 (encoding calcium-activated potassium channel, subfamily M subunit beta 4) as a possible susceptibility allele for mesial temporal lobe epilepsy (mTLE). Such a finding warrants a further well-powered study in additional carefully phenotyped cohorts. Here we examined the role of the SNP (rs398702) in a cohort of 332 patients (182 women and 150 men; mean ± SD age: 47.06 ± 18.12) who had diagnoses of mTLE. None of the patients had a mass lesion, malformations of cortical development, or traumatic brain injury. Brain MRI study revealed hippocampal sclerosis (Hs) in 86/332 (26%) patients. Most patients (254/332, 76%) patients had drug-responsive mTLE. We also enrolled 335 healthy controls (164 women and 171 men; mean ± SD age: 48.20 ± 21.90), matched for age, sex and ethnicity. All patients and controls were Caucasian and were born in Italy. The genotype distribution of the SNP rs398702 in patients and controls was within Hardy—Weinberg equilibrium ($p > 0.05$). There was no statistically significant difference in the genotype or allelic frequencies between patients and controls ($p = 0.878$ and $p = 0.666$ respectively). Moreover, such a variant did not influence the main clinical characteristics of mTLE, the presence of Hs or responsiveness to antiepileptic drugs. In conclusion, our data suggest that the rs398702 variant in the KCNMB4 gene is unlikely to influence significantly the risk of developing mTLE or its severity. They further highlight the importance of replication to confirm the validity of association study results.

Introduction

Temporal lobe epilepsies (TLEs) are the most common forms among epilepsies with focal seizure onset. Mesial temporal lobe epilepsy (mTLE), one type of TLE that is frequently associated with mesial temporal sclerosis (Engel, 1996), is

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a heterogeneous, polygenic, and complex disorder in which various susceptibility genes and environmental factors are believed to act together to produce the specific phenotype (Gambardella et al., 2009).

Association studies may help to dissect the genetic basis of these complex disorders (Cardon and Bell, 2001). Unfortunately, association studies have not produced consistent results; initial positive associations are often contradicted by later negative replication studies (Cardon and Bell, 2001). Therefore, a positive association warrants a further well-powered study in additional carefully phenotyped cohorts.

A recent study illustrated that a tagging single nucleotide polymorphism (SNP) rs398702 located 3′ of KCNMB4 (encoding calcium-activated potassium channel, subfamily M subunit beta 4) may confer susceptibility to mTLE (Cavalleri et al., 2007). Such a gene is an excellent candidate gene to mTLE, as it pertains to the large-conductance voltage- and calcium-activated potassium channels that are critical for neuronal excitability, especially in the hippocampus (Petrik et al., 2011).

Since the effects seem to be population specific, the role of any genetic variant identified in one population may not translate to another, so replication attempts should be also undertaken in cohorts ethnically different to those in which the original effect was reported. Therefore, to better assess the role of this gene in mTLE, we tried to replicate the association of the rs398702 variant with mTLE in a case—control study of 332 patients with mTLE of Italian ancestry.

Materials and methods

Patients

Data and evaluation procedures on our mTLE patients have been reported in greater detail elsewhere (Gambardella et al., 2003; Labate et al., 2006, 2008). Subjects included in this study were 332 patients (182 women and 150 men; mean ± SD age: 47.06 ± 18.12). In each patient, the diagnosis of mTLE was made on the basis of a range of clinical seizure semiology, typical mesio-temporal auras, interictal and ictal EEG, and MRI criteria. Any suggestion of seizure onset outside the mesial temporal structures, by semiology or EEG findings, was an exclusion criterion. None of the patients had a mass lesion (tumor or vascular malformation), malformations of cortical development, or traumatic brain injury. The only accepted MRI sign was hippocampal sclerosis (Hs), which was based on the characteristic MRI pattern of abnormalities (Jackson et al., 1994). Neurological examinations were unremarkable in all patients. None of our patients had mental retardation. Brain MRI study revealed evidence of hippocampal sclerosis (Hs) in 86/332 (26%) patients. We also enrolled 335 healthy controls (164 women and 171 men; mean ± SD age: 48.20 ± 21.90), matched for age, sex and ethnicity. All patients and controls were Caucasian and were born in Italy. Patients and controls gave written informed consent prior to participation in the genetic studies.

Molecular methods

Genomic DNA was extracted from venous blood using standard procedures. Genotyping was performed with TaqMan SNP Genotyping Assays (KCNMB4 rs398702: C_1017690_10) on an ABI Prism 7900HT Real Time PCR System (Applied Biosystems, Foster City, CA, USA). Assay conditions were in accordance with manufacturers' protocols, and allelic discrimination was carried out using the SDS software, version 2.2.2 (Applied Biosystems).

Power calculation

The statistical power required to achieve statistically significant associations were carried out with version 3.1 of G*Power program (Faul et al., 2007), URL: http://www.psycho.uni-duesseldorf.de/abteilungen/aap/gpower3/. The estimated effect size was calculation based on the present study size (335 for control group and 332 for case group) using a two independent sample test with a power of 80% at two-sided type I error rate 0.05. The analysis of minimal statistical power was performed post hoc with the G*Power program.

Statistical analysis

Genotype and allele frequencies, and Hardy—Weinberg equilibrium tests of the KCNMB4 SNP rs398702 were performed separately among cases and controls using the Chi-square test (χ^2 test). χ^2 analysis was used to test significance of differences in allele and genotype frequencies in controls versus affected subjects. In all tests, a p value below 0.05 was considered significant.

Results

The genotype distribution of the SNP rs398702 evaluated in this study among the patients and controls were within Hardy—Weinberg equilibrium ($p > 0.05$). We examined the allele and genotype frequencies of the KCNMB4 SNP rs398702 in 332 mTLE patients and an Italian population control group (Table 1). We did not observe a statistically significant difference in the genotype or allelic frequencies between patients and controls, as shown in Table 1 ($p = 0.878$ and $p = 0.666$ respectively). In the second step of the study, we observed that the SNP rs398702 did not influence age at seizure onset or duration of epilepsy. There was also no overrepresentation of this SNP in patients with MRI evidence of Hs. Also, we investigated the relationship between this variant and response to antiepileptic drugs, stratifying the patients into two groups: 83 patients with drug-resistant and 241 patients with drug-responsive mTLE. For the latter analysis, we excluded 8 patients for whom their last follow-up was not available. We did not observe differences between groups regarding the responsiveness to antiepileptic drugs (data not shown).

Discussion

This study has examined one of all positive genetic associations reported by Cavalleri et al. (2007) on sporadic forms of TLE in a single patient cohort, in particular we examined the contribution of the SNP rs398702 in the KCNMB4 gene to the susceptibility in mTLE. The results presented here suggest that the reported association for mTLE is not reproducible,