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CASE REPORT

GABABR1 (G1465A) gene variation and temporal lobe epilepsy controversy: New evidence

Marcelo Andrés Kauffman*, Estrella Mariel Levy,
Damian Consalvo, José Mordoh, Silvia Kochen

Centro de Epilepsia, Hospital Ramos Mejia, Buenos Aires, Argentina

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KEYWORDS

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Summary The G1465A polymorphism in the gene of the GABA type B receptor subunit 1 (GABABR1) has been linked to the risk for temporal lobe epilepsy (TLE). However, six replication studies did not show significant association between the G1465A GABABR1 gene variant and TLE. The authors examined this association in a sample of 102 patients with mesial TLE with hippocampal sclerosis (MTLE-HS) and 71 controls. The genotype distribution varied significantly between patients and controls. Heterozygous carriers of the A-allele had a 10-fold increase in risk for MTLE-HS (OR 10.01; 95% CI 3.98–25.18, $p = 3.788E-08$).

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Introduction

The syndrome of mesial temporal lobe epilepsy with hippocampal sclerosis (MTLE-HS) is considered an acquired disorder. Nevertheless, recent evidence indicates that genetic factors are important in the genesis of this syndrome.¹

GABA is the main inhibitory aminoacid of the central nervous system. Its action, mediated through GABA type B receptors, could be important in the epileptogenesis of MTLE-HS.²

An association between the G1465A polymorphism of the gene coding for the GABA type B receptor

subunit 1 (GABABR1) and temporal lobe epilepsy was reported in 2003.³ Nevertheless, six recent reports have failed to confirm this association, raising controversy about the real role of this polymorphism in the susceptibility to develop the disease.^{4–9}

The aim of the present study, which started in 2003, was to examine the association between the G1465A GABABR1 gene single nucleotide polymorphism and the MTLE-HS susceptibility in a population of Argentinean patients with MTLE-HS. We also included an exploratory analysis in other phenotypic subgroups.

Materials and methods

Between August 2003 and July 2005, we recruited 102 consecutive MTLE-HS patients from the Epilepsy Clinic at the Neurology Division of the Ramos Mejia Hospital in Buenos Aires, Argentina. The study was

* Corresponding author at: Neurogenetic Clinic, Neurology Division, Centro de Epilepsia, Hospital Ramos Mejia, Urquiza 609 (1221), Buenos Aires, Argentina. Tel.: +54 11 41270280.

E-mail addresses: marcelokauffman@gmail.com (M.A. Kauffman), elevy@leloir.org.ar (E.M. Levy), dconsalvo@fibertel.com.ar (D. Consalvo), skochen@mail.retina.ar (S. Kochen).

reviewed and approved by the local ethics committee and written informed consent was obtained from each patient prior to any sample recovery. All patients had a comprehensive diagnostic evaluation, including detailed seizure history and neurological examination, neuropsychological testing, optimized MRI study, and surface EEG. Long-term video-EEG monitoring was performed in a subset of patients. Data from all these patients was consistent with the diagnosis of typical MTLE-HS. Seventy-one healthy unrelated subjects were selected as controls. Selection of the controls was based on the lack of any positive antecedent for a seizure event or other neurologic or psychiatric disorder. Cases and controls were interviewed about ethnic background. We paid special attention to the nationality of parents and grandparents of the subjects included. Control subjects were matched for age, gender, ethnic origin (nationality of parents and grandparents), and area of residence.

The following clinical features were analyzed: age, sex, age at onset of epilepsy, prior history of febrile seizures, positive family history for epilepsy and febrile seizures, clinical semiology of seizures, seizure frequency, and response to pharmacological treatment.

Genomic DNA was isolated from whole blood using a Flexigene kit, as described by the manufac-

turer (Qiagen, Hilden, Germany). The G1465A GABABR1 polymorphism was genotyped by PCR-RFLP, as previously described.³ The PCR and RFLP reactions were performed in a blinded manner to clinical features, in batches containing equal number of samples from patients and controls.

The Hardy–Weinberg equilibrium in the cohort of healthy controls was tested using exact test. Categorical variables were compared with the χ^2 -test or Fisher exact test. We calculated ORs as measures of effect. An α level (p -value) of <0.01 was considered as significant, as standardized for genetic association studies. All p -values were two-tailed. Differences in quantitative variables were assessed with the Mann–Whitney U test, since data did not have a normal distribution. All statistical analyses were performed using the SPSS version 10.0 for Windows.

Results

The distribution of genotypes and allele frequencies of the G1465A polymorphism in exon 7 of the human GABABR1 gene in patients and controls is summarized in Table 1. Heterozygous carriers of the A allele had a 10-fold increased risk for MTLE-HS when compared to homozygous carriers of the G allele (GG vs. GA: OR 10.01; 95% CI 3.98–25.18 $p = 3.788E-08$). The

Table 1 Genotype frequencies in populations studied

Genotypes	MTEHS, $N = 102$ (%)	Controls, $N = 71$ (%)	OR (95% CI)	p -Value
A/A	0	0	NA	3.788E-08 ^a
A/G	49 (48)	6 (8.5)	10.016 (3.98–25.18) ^b	
G/G	53 (52)	65 (91.5)	1 (reference group) ^b	

NA: not applicable; MTEHS: mesial temporal lobe epilepsy with hippocampal sclerosis.

^a χ^2 -test.

^b Crude odds ratio.

Table 2 Baseline characteristics of patients with MTEHS in the present study, compared with GABBR1 G1465A association studies cohorts reported in the literature

Study	n	Male, n (%)	HS, n (%)	Age at onset, year, mean (S.D.)	FH, n (%)	FC, n (%)	Medically refractory, n (%)
Stogmann et al.	188	78 (41.5)	112 (59.6)	15.1 (13.5)	48 (25.5) ^a	42 (22.3) ^b	175 (93.1)
Jin et al.	112	64 (57.1)	67 (59.8)	16.46 (8.99)	NR	28 (25)	NR
Salzmann et al.	110	47 (42.7)	108 (98.2)	15.05 (5.23)	22 (20)	60 (54.5)	110 (100)
Cavallieri et al.	245	NR	NR	NR	NR	NR	NR
Tan et al.	234	96 (41)	140 (59.8)	15 (13.5)	NR	82 (35)	159 (68)
Ma et al.	120	49 (40.8)	NR	22.8 (10.08)	66 (55)	120 (100)	23 (19.2)
Gambardella et al.	141	63 (44.7)	31 (22)	30.9 (21.9)	51 (36.2)	21 (14.9)	14 (9.9)
Kauffman et al.	102	51 (50)	102 (100)	16.4 (13.37)	18 (17.6)	20 (19.6)	60 (58.8)

NR: no reported; FH: family history positive for epilepsy or febrile convulsions; FC: antecedent of febrile convulsions.

^a 176 patients investigated, in 12 patients clinical details regarding family history could not be assessed unambiguously.

^b 184 patients investigated, in 4 patients reliable information could not be obtained.

observed genotype distributions in the cohort of controls and the respective theoretical distributions inferred from the observed allele frequencies were not significantly different (Hardy–Weinberg equilibrium). We did not examine other genetic variants.

Baseline characteristics of our patients compared with the clinical characteristics of the different cohorts where this GABABR1 gene variant was investigated are summarized in Table 2.

In the exploratory analysis, we found that A/G genotype was not associated with either a family history of epilepsy or febrile seizures. Furthermore, we did not find an association between this genotype and severity of epilepsy, as measured by seizure frequency, clinical semiology of seizures, and response to pharmacological treatment (our cohort is formed by a 60% of medically refractory subjects) or ethnic origin of subjects included.

Discussion

The association between temporal lobe epilepsy and the GABABR1 G1465A polymorphism was originally reported by Gambardella et al. in a cohort of Italian patients that included a small number of patients with hippocampal sclerosis.³ Our results, obtained from an independent population, confirm the original findings. Although our estimate of the size effect is of a smaller magnitude than the one reported in the first study, the prevalence of G1465A polymorphism in our patients is greater suggesting a more homogeneous population.

Nevertheless, six recent studies failed to find an association between G1465A polymorphism and temporal lobe epilepsy in groups of patients with different ethnic origins. The G1465A polymorphism was not associated with a risk to develop temporal lobe epilepsy, irrespective of the presence or absence of hippocampal sclerosis, in studies performed in the United States, France, Great Britain, Australia, China, and Austria. Ethnic differences may play a role in conflicting results of genetic association studies. These differences could be due to linkage disequilibrium of the disease causing allele with different alleles at the marker locus in different populations. Alternatively, allelic heterogeneity among different ethnic groups, or other genetic or environmental factors modifying the association could explain differences among studies.¹⁰ Since our cohort is similar on clinical grounds with the ones mentioned above (see Table 2), the differences observed could depend on ethnic factors. In the XIX and XX century, Argentina received a large number of immigrants, being Italy the main source of this flow. Furthermore, a population

genetic study performed in the city of Buenos Aires showed that the genetic contribution of Europeans to the gene pool of Buenos Aires could be estimated at 67.55%.¹¹ We think that our positive finding might be due to this ethnic similarity with Gambardella's population as a consequence of our particular migratory history.

Alternatively, we must consider the possibility of false positive results in our study. Several authors have suggested possible reasons for inconsistency in the results of genetic association studies.¹⁰ One of the most common causes for failure to reproduce initial findings is population stratification.⁶ However, this can represent an important bias only under extreme situations, such as populations with few ethnic groups with large differences in disease and genotype frequencies among them.¹² Moreover, simulation studies performed in highly admixed populations, like ours, also conclude that bias as a result of population stratification is highly unlikely, even under unrealistic scenarios such as two ethnicities structured with marked differences in disease rates between them.¹² We think that population stratification is not an important factor in our findings because our population is composed by more than two ethnic groups with no large ethnic differences of temporal lobe epilepsy prevalence reported.

Another explanation for false positive findings is chance.¹⁰ In order to control for this factor some researchers suggested accepting only highly conservative *p*-values to reject the null hypothesis of no association. The most stringent threshold value should be 5×10^{-8} .¹³ We think that the level of significance in our study (4×10^{-8}) makes unlikely that our positive findings could be due to chance. The possibility of biased genotyping errors is very unlikely because all genotyping probes were performed blindly in batches with equal number of cases and controls. Since our diseased cohort genotype distribution deviated from Hardy–Weinberg equilibrium, the mode of inheritance seems to be additive.¹⁴ Furthermore, this situation might be supporting evidence for the correlation between genotype and disease since unreported or weak associations can be detected by calculating the HWE, even when statistically significant differences between genotype distributions is not present.¹⁵

Allelic variants of GABABR 1 gene are plausible risk factors for MTLE-HS development. Studies performed in animal models and humans have revealed different roles for GABAergic neurotransmission in temporal lobe epileptogenesis. Mice lacking the GABABR 1 subunit, although viable, exhibit spontaneous seizures, hyperalgesia, hyperlocomotor activity and memory impairment.¹⁶ Moreover, these mice

exhibited exaggerated in vitro epileptiform activity caused by both acute and chronic consequences of the loss of GABAB receptor function in vivo. Specifically, enhancement of *N*-methyl-d-aspartate (NMDA) receptor triggered synaptic processes, arising from the loss of the GABAB receptor-mediated inhibitory postsynaptic potential (IPSP, together with a possible promotion of depolarizing IPSPs due to the removal of GABAB auto receptor function) likely underlying these effects.¹⁷ Furthermore an increased expression of GABABR 1 and GABABR 2 receptors was found in the hippocampus of patients suffering from MTLE-HS.^{2,18} These findings could be indicating compensatory mechanisms for an enhanced neuronal hyperexcitability, present in these patients, by the inhibition of presynaptic glutamatergic neurotransmission. Moreover, since a subtle malformation of cortical development could be implicated in the pathogenesis of MTLE-HS,¹⁹ it is worthy to note the recognized role of GABA B neurotransmission in the maturation and organization of cortex and synaptic transmission.^{20,21}

Nevertheless, GABA B neurotransmission was implicated in the pathogenesis of other epileptic syndromes, as well. For example, the injection of a GABA B receptor agonist into the ventrobasal thalamus or reticular nucleus of a rat model with spontaneous absence seizures exacerbates the seizures. By contrast, injection of a GABA B receptor antagonist into the same regions suppresses the spike and wave discharges.²² However, the polymorphism here investigated and other allelic variants in GABABR 1 have not been previously found associated with the development of absence epilepsy.²³ Nevertheless, different allelic variants in other GABA receptors genes have been recognized as risk factor for absence epilepsy²⁴ and other epilepsy syndromes.²⁵

In summary, our results indicate that the polymorphism G1465A of the GABABR1 gene could be a genetic risk marker for the development of mesial temporal lobe epilepsy in certain populations. Alternatively, we cannot exclude the fact that the association we found could depend on the effect of other unknown genetic variants in the GABABR1 gene in linkage disequilibrium with the polymorphism under investigation in our population.

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