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BDNF Val66Met polymorphism is not related with temporal lobe epilepsy caused by hippocampal sclerosis in Brazilian population

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ABSTRACT

Purpose: Some variants of the brain derived neurotrophic factors (BDNF) gene, namely the Val66Met (rs6265), may contribute the risk for epilepsy development. We aimed to investigate if this polymorphism was associated with the risk for epilepsy development in TLE-HS and its correlation with epilepsy-related factors and the presence of psychiatric disorders.
Methods: We assessed 119 patients with unequivocal TLE-HS and 112 healthy controls. Individuals were genotyped for the polymorphisms of the gene encoding BDNF Val66Met.
Results: There was no difference between TLE-HS and healthy controls, for the genotypic distribution ($p = 0.636$) and allelic distribution ($p = 0.471$). There was no correlation between Val66Met and epilepsy-related factors and for psychiatric comorbidities ($p = 0.888$).
Conclusions: Our findings demonstrated that polymorphism Val66Met is not associated with TLE-HS, epilepsy-related factors and psychiatric comorbidities in this selected group of patients.

1. Introduction

BDNF (brain derived neurotrophic factor) is a neurotrophic protein involved in synaptic plasticity and survival of important neurons in the central nervous system. BDNF regulates neuronal morphology, synaptogenesis, and neuroprotective effects in diverse areas of the CNS during development, though the long-term effects of BDNF on the development of epileptogenesis in the adult brain remain controversial [1]. The major hypotheses for the functional effects of insult-induced neurotrophin changes are protection against neuronal damage and stimulation of sprouting and synaptic reorganization, therefore, a BDNF gene polymorphism may represent a genetic marker that indicates an enhanced susceptibility to seizures in patients with such a genetic predisposition, as demonstrated in studies with focal and drug-resistant epilepsy [2].

Although, temporal lobe epilepsy with hippocampal sclerosis (TLE-HS) is the most common cause of drug-resistant focal epilepsy in adults [4], there are few studies on the association of BDNF and this type of epilepsy [5–7]. These studies, however, have addressed heterogeneous groups of patients, regarding etiology, not only patients with HS. BDNF secretion and hippocampal function may be influenced by the Val66Met polymorphism [8]. Patients with TLE-HS differs from other patients with TLE in several aspects, such as clinical, electroencephalographic, outcome, and history of previous injuries. Shen et al. [6], analyzing Val66Met in a subgroup of 116 patients with TLE-HS, found that these patients genotype differed from others. Therefore, it is reasonable to state that these patients have to be assessed separately from others.

Based on this scenario, the aim of our study was to investigate whether the BDNF polymorphism Val66Met was associated with an increased risk for the development of epilepsy caused by TLE-HS, associated with epilepsy-related factors and presence of psychiatric comorbidities in a large sample of patients.

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Table 1
Demographic characteristics; genotypic and allelic distribution of the evaluated polymorphisms of BDNF (Val66Met).

Demographic characteristics	HC		TLE-HS	
	Mean	SD	Mean	SD
Age	32.57	11.11	38.49	13.73

Demographic characteristics	HC		TLE-HS		
	n	%	n	%	p-value
Gender					
Female	57	50.4	68	57.1	0.306[a]
Male	56	49.5	51	42.9	
Ethnicity					
Caucasian	68	60.2	87	73.1	0.056[b]
African	24	21.2	17	14.3	
African-descent	17	15	15	12.6	
Asian	4	3.5	0	0	
Genotype					
Met\Met	3	2.7	2	1.8	0.636[b]
Val\Met	22	19.8	28	24.8	
Val\Val	86	77.5	83	73.5	
Allele					
Met	27	12.3	33	14.6	0.471[a]
Val	193	87.7	193	85.4	

BDNF: brain derived neurotrophic factor; HC: healthy controls; Met: methionine; N: number TLE-HS: temporal lobe epilepsy with hippocampal scleroses, Val: Valine.
[a] Pearson's Chi-squared test.
[b] Fisher's exact test.

2. Methods

2.1. Participants

Patients with TLE-HS and healthy controls (HC) who participated signed an informed consent form approved by the local ethics committee (45168915.1.0000.0068). We determined the ethnicity of each participant through a self-reported data about their four grandparents' ancestry.

2.1.1. Patients with TLE-HS

We included patients diagnosed with TLE-HS, classified according to the ILAE criteria. Based on these, we included 119 patients with TLE-HS as demonstrated in Table 1. We obtained epilepsy-related factors with a detailed questionnaire, file records, EEGs, and Video-EEGs.

2.1.2. Healthy controls

We recruited HC from the general population, with no history of epilepsy and psychiatric comorbidities. Controls underwent a neurological interview followed by a physical and neurological examination. The SCID and DSM-IV-TR was used for the psychiatric evaluation as demonstrated in Table 1.

2.2. Genotyping

Genomic DNA was extracted by the salting-out method from peripheral leukocytes. DNA was quantified by spectrophotometry using a NanoDrop®.

BDNF_rs6265 polymorphism was amplified out using: GoTaq Probe qPCR Master Mix (Promega®) 1 µl (1x), genotyping assay for SNP TaqMan®. Primer sequences ATCATTGGCTGACACTTTCGAACAC A/G TGATAGAAGAGCTGTTGGATGAGGA (Exxtend®) 1 µl (1x), betaine 0.5 M/µl (Sigma®), DNA genomic 10 ng/µl.

The discrimination allelic was performed using Line Gene 9600 (BIOER Technology CO), amplification and fluorescence curve before and after (45 cycles, 15 s at 95 °C and 1 min 60 °C).

2.3. Statistical analysis

Data are presented as mean and standard deviation for numerical variables or absolute and relative frequencies for categorical variables. We compared groups on continuous quantities by Brunner-Munzel test (two groups) or Kruskal-Wallis test (multiple groups) and factors with Pearson chi-square test or Fisher's exact test. All analyses were conducted on R 3.4.1 and type error set at 5%.

3. Results

There were no differences between groups considering gender ($p = 0.306$) and ethnic group ($p = 0.056$).

3.1. BDNF and TLE-HS

Patients and controls did not differ for the Val66Met (rs6265) genotypic polymorphism of BDNF ($p = 0.636$) and allelic distribution ($p = 0.471$), as demonstrated in Table 1.

3.2. BDNF and epilepsy-related variables

There was no correlation epilepsy-related factors and between Val66Met and epilepsy-related factors, as shown in Table 2.

3.3. Psychiatric comorbidities in TLE-HS

There was no correlation between Val66Met and the presence of psychiatric comorbidities ($p = 0.888$) and family history of psychiatry disease ($p = 0.612$) (Table 2).

4. Discussion

This is the first study addressing the influence of genetic variants of the BDNF Val66Met in a large sample of patients with TLE-HS. Although it is not feasible to obtain a homogeneous sample in clinical studies, considering clinical factors, we evaluated patients with the same etiology - HS. There are no previous studies about BDNF Val66Met in TLE, determined by HS. Since patients with TLE-HS present a particular phenotype, it is reasonable to state that the genotype also differ from patients with other etiologies (e.g., tumors, malformations of cortical development and gliosis). In addition, BDNF has a direct impact on neuronal growth and plasticity in hippocampus and amygdala networks [9] with the Met allele associated with a functional decrease in activity-dependent secretion of BDNF, and the Val allele is associated with increased synaptic plasticity and growth [8].

The ethnic diversity, environmental factors, and sample size can challenge the identification of genetic variants related to epilepsy. These factors may also be responsible for the difficulties to reproduce results obtained in previous studies [3,5–7]. However, a study surprisingly found a more homogeneous ancestry throughout Brazil than previously estimated, with 60–78% of European background [10]. Besides, the South and Southeast regions of Brazil present a higher concentration of European ancestry, as already discussed in a previous paper [11]. Based on this, our sample is more homogeneous than previously thought.

The study of Shen et al. [6] showed that the frequency of Met allele was found to be lower in patients with TLE compared with controls, and the frequency of Met66 allele carriers was significantly lower than those non carriers in patients with TLE-HS. In our series, we were unable to strengthen the evidence that BDNF Val66Met was associated with the risk for epilepsy development. We found similar frequencies of the genotype and allelic distribution in patients with TLE-HS and HC. Both studies addressed a similar number of patients with TLE-HS with