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Tryptophan hydroxylase 2 (*TPH2*) gene polymorphisms and psychiatric comorbidities in temporal lobe epilepsy

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ABSTRACT

Psychiatric comorbidities are frequent in temporal lobe epilepsy (TLE). It is plausible that variance in serotonin-related genes is involved in the susceptibility of these associations. We report here the results on the association of *tryptophan hydroxylase 2* (*TPH2*) gene polymorphisms with psychiatric comorbidities in TLE. A cohort study was conducted on 163 patients with TLE. We assessed the influence of the rs4570625 and rs17110747 polymorphisms in the *TPH2* gene on psychiatric comorbidities in TLE. In patients with TLE, the presence of the T allele in the rs4570625 polymorphism was associated with psychotic disorders (OR = 6.28; 95% CI = 1.27–17.54; $p = 0.02$), while the presence of the A allele in the rs17110747 polymorphism was associated with alcohol abuse (OR = 20.33; 95% CI = 1.60–258.46; $p = 0.02$). Moreover, we identified male gender (OR = 11.24; 95% CI = 1.68–76.92; $p = 0.01$) and family history of psychiatric disorder (OR = 15.87; 95% CI = 2.46–100; $p = 0.004$) as factors also associated with alcohol abuse in TLE. Conversely, a family history of epilepsy was inversely associated with alcohol abuse (OR = 0.03; 95% CI = 0.001–0.60; $p = 0.02$). *Tryptophan hydroxylase 2* gene allele variants might be risk factors for psychiatric conditions in TLE. More specifically, we observed that the T allele in the rs4570625 polymorphism was associated with psychotic disorders, and the A allele in the rs17110747 *TPH2* polymorphism was associated with alcohol abuse in patients with TLE. We believe that this study may open new research venues on the influence of the serotonergic system associated with psychiatric comorbidities in epilepsy.

1. Introduction

Epilepsy is a chronic disorder that affects people of all ages and social levels. It comprises a group of chronic neurological disorders characterized by recurrent unprovoked seizures, resulting from transitory impairment of brain function due to abnormal neuronal excitability and/or synchronization [1]. Subjects with epilepsy have a greater risk of developing neuropsychiatric comorbidities, especially those with temporal lobe epilepsy (TLE). The main psychiatric disorders involved in TLE are depression, anxiety, and psychosis, with prevalences ranging from 11 to 44%, from 15 to 25%, and from 2 to 8%, respectively [2–5].

Evidence suggests that the association of psychiatric disorders with epilepsy might be related to common biological substrates [6–8]. A growing body of evidence supports the role of the neurotransmitter serotonin (5-hydroxytryptamine, 5HT) in the regulation, development, propagation, and maintenance of seizures [9–12]. In general, preclinical and clinical studies demonstrate an inverse correlation between extracellular brain 5HT levels and susceptibility to seizures, although exceptions have also been described [13]. On the other hand, a large body of evidence shows that low concentrations of serotonin metabolites in plasma and cerebrospinal fluid impair the neuroendocrine responses on stimulation of serotonergic receptors and induce the return of depressive symptoms after successful antidepressant drug treatment, which supports the hypothesis that serotonergic activity is impaired in depression. All effective drugs available for the treatment of depression increase the activity of serotoninergic systems [14–16].

Brain 5HT availability depends on various factors, including the gene encoding the 5HT transporter (5HTT), a cell membrane protein responsible for the clearance of 5HT from the synaptic cleft [17], and monoamine oxidase A (MAO-A), a mitochondrial enzyme responsible for the degradation of 5HT and of other monoamine neurotransmitters [18]. We have previously shown that the C-1019G polymorphism in 5-HT1A was associated with anxiety disorders in patients with TLE [19]. These results opened a precedent to explore the possible role of genetic variants of other serotonergic system-related genes and their involvement on psychiatric comorbidity in patients with epilepsy.

A recently published meta-analysis [20] detected a strong correlation between the rs4570625 polymorphism of the *tryptophan*

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hydroxylase 2 (*TPH2*) gene and major depressive disorder. Tryptophan hydroxylase 2 is highly expressed in the raphe nuclei of the midbrain, where it is a rate-limiting enzyme in serotonin synthesis. Tryptophan hydroxylase 2 polymorphisms have been associated with some neuropsychiatric disorders [20–22]. Therefore, it is plausible that polymorphisms of the *TPH2* gene could also modify behavioral traits in patients with epilepsy. In this study, we investigated the association between genetic variants of the *TPH2* gene and psychiatric comorbidity in patients with TLE.

2. Methods

2.1. Patients

A cohort study was conducted on 163 consecutive patients of Western European (white) descent, diagnosed with TLE at the Epilepsy Outpatient Clinic of the Hospital de Clínicas de Porto Alegre (HCPA). Inclusion criteria were based on the 1989 ILAE electroclinical classification [23] and neuroimaging results. Specifically, we included patients presenting with typical complex focal seizures, with or without generalization, with interictal EEG showing anterior temporal sharp waves and compatible neuroimaging. Patients with extratemporal epilepsies, mental retardation, and systemic diseases were excluded. The study was approved by the Ethics Committee of our institution and is in accordance with the Declaration of Helsinki. All subjects included provided written informed consent to participate in the study.

2.2. Psychiatric interview

All patients were assessed by means of the Structured Clinical Interview for DSM-IV (SCID) [24], divided into six modules, for the detection of one or more lifelong diagnoses from the Axis I Diagnostic and Statistical Manual, fourth edition (DSM-IV) [25]. The Structured Clinical Interview for DSM-IV detected Axis I psychiatric diagnoses in 102 (62.6%) of the 163 patients with TLE (group with TLE with psychiatric comorbidity). In 61 (37.4%) of the 163 patients with TLE, SCID showed negative results (group with TLE without psychiatric comorbidity). These two groups of patients (group with TLE with psychiatric comorbidity and group with TLE without psychiatric comorbidity) were compared for clinical and genotypic differences.

2.3. Genotyping

Deoxyribonucleic acid was extracted from peripheral blood leukocytes by the salt precipitation method [26]. Subjects were genotyped for the rs4570625 and rs17110747 polymorphisms in the *TPH2* gene [27]. Polymerase chain reaction was performed using 20 ng of DNA in SNP Genotyping Assays provided by Applied Biosystems (Foster City, CA, USA). The procedure that followed the protocol for Taqman SNP genotyping was also provided by Applied Biosystems with the use of Taqman Genotyping PCR MasterMix (Applied Biosystems, Foster City, CA, USA). Each assay consisted of unlabeled forward and reverse primers and two reporters that were dye-labeled with FAM and VIC and were designed for allelic discrimination of specific polymorphisms. Both alleles were scored in a single well by measuring the fluorescence at the end of the PCR reaction.

2.4. Statistical analysis

Categorical variables were compared by using the two-tailed Pearson chi-squared test and Fisher's exact test. Numerical variables were compared by the independent Student *t*-test, utilizing the Levene test for analysis of equality of variance. All statistical analyses were carried out using the IBM® SPSS® Statistics Package v. 20. Logistic regression was used to examine the independent effect of each variable. To determine the number of independent variables to be included in our logistic regression model, we used the parameters suggested in the literature [28–30]. Results were reported as odds ratio (95% confidence interval) and were considered significant if p was equal to or lower than 0.05.

3. Results

Of the 163 patients with TLE, 57 (35%) were men, and 106 (65%) were women. Psychiatric disorders were observed in 102 (62%) of the patients studied. In our sample, alcohol abuse was observed in 9 (5.5%) patients, 7 (77.8%) of them were male.

The mean ages of patients with epilepsy with psychiatric disorders (group with TLE with psychiatric comorbidity) and those without psychiatric disorders (group with TLE without psychiatric comorbidity) were 43.4 (SD = 12.7) years and 45.3 (SD = 12.3) years, respectively, with no significant difference between them ($p = 0.34$). Most (70.6%) of the patients in the group with TLE with psychiatric comorbidity were women, whereas in the group with TLE without psychiatric comorbidity, 55.7% were women, and 44.3% were men, a trend towards a significant gender difference ($p = 0.05$). A family history of psychiatric disorders was found in 39.2% of the patients in the group with TLE with psychiatric comorbidity, and in 23% of the patients in the group with TLE without psychiatric comorbidity, a statistically significant difference ($p = 0.03$). The age of onset and duration of disease, the mean seizure frequency, the presence of hippocampal lesion, a left-sided epileptogenic focus, the number of antiepileptic drugs used, a family history of epilepsy, seizure control, interictal EEG activity, the presence of initial precipitating injury, and the use of benzodiazepine (BZD) did not differ between patients with TLE with and without psychiatric comorbidities. The clinical and demographic characteristics of patients are presented in Table 1.

The genotype distribution of the rs4570625 and rs17110747 polymorphisms of the *TPH2* gene according to psychiatric disease in patients with TLE is summarized in Table 2. We found that there was no significant association between these polymorphisms and the presence of neuropsychiatric disorders in patients with TLE when analyzed together. However, when each psychiatric disorder was analyzed individually (mood disorder, anxiety disorder, psychosis, and alcohol abuse), the frequency of the rs17110747 polymorphism differed between patients with TLE with and without a history of alcohol abuse, with the frequency of the A allele being lower in patients with a history of alcohol abuse (33.3%) than in individuals without a history of alcohol abuse (22.7%; $p = 0.049$). Moreover, the frequency of the

Table 1
Characteristics of patients with TLE with and without psychiatric comorbidities.

Variable	All ($n = 163$)	TLE-only ($n = 61$)	TLE-psych ($n = 102$)	p
Mean age (years, SD)	44.1 (12.5)	45.3 (12.3)	43.4 (12.7)	0.34
Mean age of epilepsy onset (years, SD)	19.0 (14.6)	18.6 (14.8)	19.3 (14.5)	0.77
Mean epilepsy duration (years, SD)	25.1 (14.1)	26.7 (13.5)	24.1 (14.5)	0.26
Mean seizure frequency (seizure/month, SD)	2.82 (5.56)	2.02 (4.48)	3.30 (6.09)	0.16
Female sex	106 (65.0)	34 (55.7)	72 (70.6)	0.05[a]
Initial precipitating injury	41 (25.2)	13 (21.3)	28 (27.5)	0.38
Controlled seizures	63 (38.7)	26 (42.6)	37 (36.3)	0.42
Abnormal MRI	59 (36.2)	26 (42.6)	33 (32.4)	0.39
MTLE-HS or hippocampal atrophy	34 (20.9)	16 (26.2)	18 (17.7)	0.19
Unilateral interictal EEG	91 (55.8)	35 (57.4)	56 (54.9)	0.76
Left-sided focus	121 (74.2)	42 (68.9)	79 (77.5)	0.22
Family history of epilepsy	57 (35.0)	19 (31.1)	38 (37.3)	0.43
Family history of psychiatric disease	54 (33.1)	14 (23.0)	40 (39.2)	0.03[a]
Monotherapy	83 (50.9)	33 (54.1)	50 (49.0)	0.63
Benzodiazepine use	30 (18.4)	10 (16.4)	20 (19.6)	0.61

Values are presented as frequency (percentage) or mean (SD). TLE-only and TLE-psych = patients with temporal lobe epilepsy without and with psychiatric comorbidities, respectively. MTLE-HS is mesial temporal hippocampal sclerosis.

[a] Significant.