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Higher transcription alleles of the MAOA-uVNTR polymorphism are associated with higher seizure frequency in temporal lobe epilepsy

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ABSTRACT

Background: There is evidence of an imbalance in the neuromodulatory system mediated by serotonin (5-HT) in patients with drug-resistant temporal lobe epilepsy (TLE). This study analyzed the monoamine oxidase A promoter variable number of tandem repeats (MAOA-uVNTR) polymorphism in patients with temporal lobe epilepsy with hippocampal sclerosis (TLE-HS). Therefore, we assessed the association between this genetic variant and seizure predisposition and severity in patients with TLE-HS.

Methods: One hundred nineteen patients with TLE-HS and 113 healthy volunteers were assessed. First, we genotyped all individuals for the MAOA-uVNTR genetic polymorphism. Second, we compared patients and controls and evaluated clinical variants of epilepsy.

Results: There was no difference between the TLE-HS and control groups regarding genotypic and allelic distributions of MAOA-uVNTR polymorphism ($p = 1.000$). Higher transcription alleles of the MAOA-uVNTR were associated with higher seizure frequency ($p = 0.032$) and bilateral tonic-clonic seizures ($p = 0.016$).

Conclusions: In a selected group of patients with TLE-HS, the polymorphism MAOA-uVNTR was associated with some aspects of epilepsy severity, namely seizure frequency and bilateral tonic-clonic seizures.

1. Introduction

Temporal lobe epilepsy caused by hippocampal sclerosis (TLE-HS) is the most frequent cause of drug-resistant epilepsy in adults referred to tertiary care centers for epilepsy surgery (Engel et al.,1997). A previous review (Bagdy et al.,2007) showed an imbalance of the neuromodulatory system mediated by serotonin (5-HT) in patients with drug-resistant TLE. Savic et al. (2004), in a functional neuroimaging study using positron emission tomography (PET), showed that there was a reduction of 5-HT1A receptor binding in the epileptogenic hippocampus in patients with mesial TLE. Merlet et al. (2004), in a study using PET and intracranial recordings with stereo-electroencephalography (SEEG), demonstrated that the 5-HT1A receptor binding was reduced in patients with TLE compared to controls. Besides, there was an association between this reduction with the degree of epileptogenicity established by SEEG.

There are studies with human brain tissue - hippocampal/temporal cortical samples (Louw et al., 1989) and temporal cortex (Naffah-Mazzacoratti et al., 1996; Pintor et al., 1990) - showing an increase in the levels of 5-HT and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA) in the epileptogenic cortex, which is more evident in brain areas with more frequent spikes.

Monoamine oxidase (MAOA) is a mitochondrial enzyme responsible for the degradation of 5-HT and other monoamine neurotransmitters (Murphy et al.,1979). The MAOA isoform has high selectivity for 5-HT and is localized primarily in the brain (Westlund et al., 1993). An upstream variable number of tandem repeats (VNTR) polymorphism in MAOA gene (MAOA-uVNTR) has been shown to affect both MAOA enzyme activity and the transcriptional efficiency of the MAOA promoter (Sabol et al.,1998). The MAOA-uVNTR polymorphism can be classified as producing greater (high-MAOA) or less (low-MAOA) transcription of the MAOA gene (Newman et al.,2005). Alleles with 3.5 or 4 copies of the repeat sequence are transcribed 2–10 times more efficiently than those with 3 or 5 copies of the repeat, suggesting an

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optimal length for the regulatory region (Sabol et al.,1998), and are associated with a decrease in 5-HT concentration. A decrease in 5-HT concentration could lead to an imbalance in the neuromodulatory system, resulting in the susceptibility to the development of epileptic seizures, as previously demonstrated in human tissue (da Fonseca et al., 2015).

In this study, we aimed to evaluate the association between MAOA genetic variant MAOA-uVNTR and seizure susceptibility in a group of patients with TLE-HS compared to controls.

2. Methods

2.1. Participants

Patients and controls signed an informed consent form approved by the local Ethics Committee (number 17030713.2.1001.0068).

The study protocol and the clinical profile of our patients have already been described in our previous studies (Alcantara et al., 2018a,b; Vincentiis et al.,2018).

As published elsewhere (Alcantara et al., 2018a,b; Vincentiis et al.,2018), we included patients diagnosed with TLE-HS determined by MRI with clinical (semiology) and neurophysiological (video-EEG) data that corroborated temporal lobe seizures. We evaluated healthy volunteers, recruited from the general population, with no lifetime personal or family history of CNS disorders or psychiatric comorbidities.

Based on these criteria, we evaluated 119 patients with TLE-HS and 113 controls. There was no difference between the TLE-HS and control groups regarding gender and ethnicity (Table 1).

2.2. Genotyping

We used conventional polymerase chain reaction (PCR) to amplify regions of interest. The allele size was evaluated by capillary electrophoresis using a Fragment Analyzer™ with the DNF-910 kit. Primer F: ACAGCCTGACCGTGGAGAAG and primer R: GAACGGACGCTCCATTC GGA (Exxtend®), 100 mmol (0.05 µL); buffer (Invitrogem®), 1 µL (10x); dNTP (Invitrogem®), 1.25 mmol (1.6 µL); platinum Taq polymerase (Invitrogem®), 5 U (0.1 µL); MgCl (Invitrogem®), 50 mmol (0.3 µL); genomic DNA, 25 ng (2 µL) and ultrapure water to final volume of 10 µL. The same technique was applied in our previous work (Alcantara et al., 2018a).

Table 1
Demographics and genotypic distribution.

	TLE-HS	Controls	P
Age [Mean (SD)]	38.6 (13.8)	32.4 (11.0)	< 0.001
	TLE-HS N %	Controls N %	P
Gender			0.612
Female	68 (57.2%)	57 (50.5%)	
Male	51 (42.8%)	56 (49.5%)	
Ethnicity			0.223
Caucasian	87 (73.1%)	68 (60.2%)	
African-descent	17 (14.3%)	24 (195%)	
African	15 (12.6%)	17 (15.0%)	
Asian	0 (0.0%)	4 (5.3%)	
Genotype			1.000
High-MAOA	54 (46.5%)	57 (50.4%)	
Low-MAOA	36 (29.3%)	32 (28.3%)	
Heterozygous	29 (24.2%)	24 (21.2%)	

Fisher´s exact test.

2.3. Statistical analysis

Clinic epilepsy factors were evaluated by Fisher's exact test (age at onset, duration of epilepsy) and by Kruskal-Wallis test (laterality, seizure frequency, antiepileptic drugs – mono vs. polytherapy, the presence of bilateral tonic-clonic seizures, febrile seizures, status epilepticus, family history of epilepsy and psychiatric disorders).

All statistical analysis was carried out on SPSS for Windows Version 22.0 (SPSS Inc., Chicago, IL, USA). Type I error was set at 5%, and adjustment for multiple testing was carried out by Holm-Bonferroni Sequential Correction. We calculated the sample size with the program G Power version 3.1.9.2. For a sample of 100–150 individuals per group, the statistical power is 95%, as published earlier (Vincentiis et al., 2018).

3. Results

3.1. MAOA-uVNTR and TLE-HS

The genotypic and allelic distributions of MAOA-uVNTR did not differ between the TLE-HS and control group **(**Table 1**).**

3.2. MAOA-uVNTR and epilepsy-related variables

There was an association between the presence of high-MAOA alleles and higher seizure frequency ($p = 0.032$) and the presence of bilateral tonic-clonic seizures ($p = 0.016$) **(**Table 2**).**

4. Discussion

This study is the first addressing the influence of MAOA in a large sample of patients with TLE-HS. Patients with TLE-HS differ significantly in several clinical aspects compared to patients without HS caused by other etiologies (e.g., tumors, focal cortical dysplasias, and gliosis) (review in Coan and Cendes, 2013). Although it is not feasible to obtain a homogeneous sample in clinical studies, considering epilepsy-related factors, we evaluated patients with the same etiology – HS. For this reason, all studies performed by our group (Alcantara et al., 2018a,b; da Fonseca et al., 2015; Vincentiis et al., 2018) included a homogeneous group of patients, regarding etiology.

Regarding MAOA, patients with TLE-HS did not differ from controls. In agreement with our data, Stefulj et al. (2010), in a sample of patients with TLE caused by distinct etiologies, observed that genetic variants of MAOA-uVNTR polymorphism were equally represented among TLE patients and controls, suggesting that investigated polymorphism in this gene do not exhibit significant effects on the susceptibility to TLE.

Considering epilepsy severity, we observed that high-MAOA alleles were associated with higher seizure frequency and the presence of bilateral tonic-clonic seizures. Since MAOA is related to the inactivation of 5-HT, it is plausible that such 5-HT concentration decreases lead to an increase in neuronal hyperexcitability and a higher seizure frequency and bilateral tonic-clonic seizures in this group of patients. In human tissue, several studies with resected tissue from different regions in small samples of patients with TLE, not necessarily with HS, showed an increase of 5-HT in the temporal neocortex (Naffah-Mazzacoratti et al., 1996; Pintor et al., 1990), and hippocampal/ temporal neocortex tissue (Louw et al., 1989). In opposition to these findings, our group (da Fonseca et al., 2015), in a previous study with human tissue (hippocampus) of the same patients with TLE-HS included in this study, demonstrated a 5-HT concentration decrease associated with bilateral tonic-clonic seizures. Our findings with MAOA-uVNTR polymorphism corroborated our previous data with human tissue and suggested that this polymorphism, related to the increased degradation of 5-HT, can lead to a decrease in 5-HT concentration with an impact in seizure frequency.

It is of note that high-MAOA alleles may lead to a decrease in

Table 2
Epilepsy-related factors.

	TLE-HS	Low-MAOA	High-MAOA	Heterozygous	P[a]
Age of onset [Mean (SD)]	11.3 (10.6)	11.57 (11.93)	12.28 (10.59)	8.09 (8.38)	0.145
Epilepsy duration [Mean (SD)]	25.9 (13.0)	26.15 (14.91)	24.33 (12.22)	28.00 (12.58)	0.420
	TLE-HS N %	Low-MAOA	High-MAOA	Heterozygous	P[b]
Side of the lesion					0.064
Right	43 (36.1%)	17 (47.2%)	13 (24.0%)	13 (44.8%)	
Left	62 (52.1%)	16 (44.4%)	31 (57.4%)	15 (51.7%)	
Bilateral	14 (11.8%)	3 (8.4%)	10 (18.6%)	1 (3.5%)	
Seizure frequency					**0.032**
Daily/weekly	61 (51.3%)	15 (41.6%)	37 (68.6%)	14 (48.2%)	
Bi-weekly/montly	40 (33.6%)	16 (44.4%)	15 (27.7%)	9 (31.0%)	
Yearly/no seizures	18 (15.1%)	5 (14.0%)	2 (3.7%)	6 (20.8%)	
AED					0.917
Monotherapy	27 (22.7%)	9 (25.0%)	17 (31.4%)	5 (17.3%)	
Polytherapy	92 (77.3%)	27 (75.0%)	37 (68.6%)	24 (82.7%)	
Bilateral tonic-clonic seizures					**0.016**
Present	85 (71.4%)	23 (63.8%)	38 (70.3%)	24 (72.4%)	
Absent	34 (28.6%)	13 (36.2%)	16 (29.7%)	5 (27.6%)	
Status epilepticus					0.712
Present	23 (19.3%)	8 (24.2%)	9 (18.4%)	6 (31.5%)	
Absent	85 (71.4%)	25 (75.8%)	40 (81.6%)	18 (68.5%)	
Missing data	11 (9.3%)				
Febrile seizures					0.918
Present	29 (24.3%)	9 (29.0%)	13 (28.9%)	7 (33.3%)	
Absent	68 (57.1%)	22 (71.0%)	32 (71.1%)	14 (66.7%)	
Missing data	22 (18.6%)				
Family history of epilepsy					
Present	53 (44.5%)	19 (59.4%)	24 (48.9%)	10 (45.8%)	0.495
Absent	51 (42.8%)	13 (40.6%)	25 (51.1%)	13 (54.2%)	
Missing data	15 (12.7%)				
Family history of psychiatric disorders					0.900
Present	42 (35.3%)	11 (36.7%)	19 (39.6%)	10 (43.5%)	
Absent	61 (51.2%)	19 (63.3%)	29 (60.4%)	13 (56.5%)	
Missing data	16 (13.5%)				

[a] Fisher´s exact test.
[b] Kruskal-Wallis test.

monoamine concentration, not restricted to 5-HT. The impairment of dopaminergic system in TLE has already been demonstrated by neuroimaging studies. Bouilleret et al. showed reduced binding to dopamine transporter (DAT) in patients with drug-resistant TLE-HS (Bouilleret et al., 2008). In addition, a decreased D2/D3 receptor density in TLE (Werhahn et al., 2006) seems to have a negative correlation with epilepsy duration (Bernedo Paredes et al., 2015). Regarding the noradrenergic system, increased and decreased of norepinephrine (NE) levels could cause dysregulation of NE-dependent functions, which in turn may contribute to increased seizure susceptibility (review in Ghasemi and Mehranfard, 2018). However, the predominant effect of NE release in entorhinal cortex appears to be inhibitory (Xiao et al., 2009). Noradrenergic abnormalities are involved in increased seizure susceptibility and severity detected in genetically epilepsy-prone rats (GEPRs) (Dailey and Jobe, 1986) and NE levels (Tsuda et al., 1993), as well as the density of adrenergic receptors (Brière et al., 1986; Nicoletti et al., 1986), are reduced following seizures. Therefore, the presence of high-MAOA alleles leads to an increase in expression and enzymatic activity. Consequently, it may lead to increased metabolism of monoamines that are essential to neuronal excitability.

The relevance of MAOA for epilepsy severity has been demonstrated by its involvement in other syndromes with epilepsy and mental retardation that carries an inherited duplication (Klitten et al., 2011), or deletion (Whibley et al., 2010) of Xp11.3, including MAOA gene. In line with these findings, our study yielded that high-MAOA allele is associated with higher seizure frequency in patients with TLE-HS.

In conclusion, this study showed the association of MAOA-uVNTR with some aspects of epilepsy severity, namely seizure frequency and the presence of bilateral tonic-clonic seizures.

Disclosure of conflicts of interest

None.

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