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Epilepsy and serotonin (5HT): Variations of 5HT-related genes in temporal lobe epilepsy

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ABSTRACT

Several lines of evidence point to the role of serotonin (5HT) neurotransmission in the epileptogenesis. The present preliminary study investigated possible association of the temporal lobe epilepsy (TLE) with the polymorphisms in several 5HT-related genes, including serotonin transporter (5HTT), monoamine oxidase A (MAO-A) and serotonin receptors 5HT-1A, 5HT-1B and 5HT-2C. All participants (101 TLE patients and 170 healthy controls) were unrelated individuals of Croatian origin. 5HT-1B allele 861G was found to be slightly overrepresented in the patient group ($p = 0.0385$). No significant differences between groups were observed for the other tested polymorphisms. Within the limitations imposed by the size of our sample, negative findings suggest that the respective loci do not make considerable contribution to the etiopathogenesis of TLE. Further examination of 5HT-1B gene, which yielded positive result at a trend level, is possibly warranted.

Epilepsy 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. A growing body of evidence supports the role of neurotransmitter serotonin (5-hydroxytryptamine, 5HT) in the regulation of seizure development, propagation and maintenance. In general, studies on animal models, as well as on humans, demonstrate an inverse correlation between extracellular brain 5HT levels and susceptibility to seizures, although exceptions have also been described [1].

Brain 5HT availability depends on various factors, including the genetic ones. Of particular interest is gene encoding 5HT transporter (5HTT), a cell membrane protein responsible for the clearance of 5HT from the synaptic cleft [14]. We and others have previously shown that genetic variations in the promoter (5HTTLPR) and intron 2 (5HTTVNTR) region of the 5HTT gene influence expression of this gene, participating in the regulation of 5HT signaling [7,13]. Notably, clinical studies have associated mentioned polymorphic regions with the susceptibility to various conditions characterized by serotonergic dysfunction [23]. Another important factor in serotonin inactivation is monoamine oxidase A (MAO-A), a mitochondrial enzyme responsible for degradation of 5HT and other monoamine neurotransmitters. An upstream variable number of tandem repeats polymorphism in MAO-A gene (MAOA-uVNTR) has been shown to affect both, transcriptional efficiency of the MAO-A promoter [18] and MAO-A enzyme activity [3].

Membrane receptors for serotonin, classified into seven distinct families (5HT-1–5HT-7), are widely distributed throughout the brain [5]. Mutant mice lacking 5HT-1A [22] or 5HT-2C [2,27] receptors display lower seizure threshold and/or increased seizure activity, implicating the respective 5HT receptors in the regulation of neuronal excitability. 5HT-1A receptors act as a presynaptic autoreceptors in the raphe neurons and also as a major postsynaptic receptors in hippocampal, cortical, and hypothalamic regions [10]. 5HT-1A receptor gene contains a single nucleotide polymorphism (SNP) C-1019G in the promoter region, which was shown to influence 5HT-1A receptor expression and binding potential, as well as amygdala reactivity [12]. Gene encoding the 5HT-2C receptor, a postsynaptic receptor expressed in different brain regions, also contains functional SNP in the coding region (C68G), which results with the substitution of cysteine for serine at the position 23 (Cys23Ser) [11]. Pharmacological studies on animal models have implicated also the role of 5HT-1B receptors in the epileptogenesis [24,28]. 5HT-1B receptors are widely distributed in axons and axon terminals where they function as auto- or hetero-receptors modulating release of various neurotransmitters, including 5HT itself [21]. Coding region of the 5HT-1B gene contains a silent SNP G861C which was shown to correlate with the number of 5HT-1B receptors in human post-mortem brain cortex [8].

The current study investigated the possible association of 5HTTLPR, 5HTTVNTR, MAOA-uVNTR, 5HT-1A C-1019G, 5HT-1B G861C and 5HT-2C cys23ser polymorphisms with temporal lobe epilepsy (TLE), the most common form of the focal epilepsy. We

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have hypothesized that functional genetic variants in the candidate 5HT-related genes could impact neuronal network excitability and possibly contribute to susceptibility to epilepsy.

The study included 101 consecutive patients with TLE (mean age 32 ± 13 years; 68% females), recruited at the Zagreb Epilepsy Centre. Diagnoses of TLE were based on the comprehensive clinical and electroencephalographic (EEG) evaluations. Mean age at onset of epilepsy was 21.5 ± 14.3 years, whereas mean duration of epilepsy was 11.9 ± 10.3 years. 9.1% of patients had antecedent of febrile seizures and 4.9% had family history of seizures. Average number of antiepileptic drugs per patient was 1.6 ± 0.8. MRI data were available for 57 patients (56.4%), and 25 of them (43.8%) had hippocampal sclerosis or temporal cortex atrophy. The control group consisted of 170 healthy blood donors (mean age 37 ± 13 years; 69% females), without personal or family history of neuropsychiatric disorders. The study was approved by the Ethical Committee of the Medical Faculty, University of Zagreb. Written informed consent was obtained from each participant.

Genomic DNA was isolated from blood samples and target regions encompassing the investigated polymorphic sites were amplified by polymerase chain reaction (PCR). Detailed procedures used for genotyping polymorphisms in 5HTT [6], MAO-A [4], 5HT-1B [26] and 5HT-2C [25] genes have been in detail described previously. Genotyping of 5HT-1A receptor was performed using a slightly modified procedure described by Rothe et al. [17]. Statistical analyses were carried out using GraphPad InStat (version 3.01) software. Differences in genotype distributions between case and control groups, as well as the presence of Hardy-Weinberg equilibrium, were tested by the two-sided χ^2 test for independence. Two-sided Fisher's Exact Test (FET) was used for the comparison of allele frequencies. Odds Ratio (OR) with 95% confidence interval (95% CI) was calculated using the approximation of Woolf. The level of significance was set at 0.05 (no correction for the multiple testing was applied because of the exploratory approach to a complex disorder for which a phenotype-genotype relationship has not been established [16]).

Genotype and allele distributions of the investigated polymorphisms in TLE patients and control subjects, as well as the corresponding p values for the differences between the groups, are presented in Table 1. Genotype frequencies of all polymorphisms in both groups accorded with Hardy-Weinberg equilibrium (p values >0.05). No significant differences between the groups were observed for genotype distributions of any of the investigated polymorphisms (Table 1). 5HT-1B allele 861G was found to be overrepresented in the patient group (p = 0.0385, OR = 1.574, 95% CI = 1.031–2.402), while alleles of other genes were similarly distributed among controls and cases (Table 1).

Association of 5HTTLPR polymorphism with the idiopathic generalized epilepsy (IGE) [20] and TLE [15] has been previously investigated in the German (133 patients) and Italian (276 patients) population, respectively. Both studies, in line with our present results (101 patients), reported the lack of association. 5HTTVNTR polymorphism was also not associated with TLE in our and one previous study [9], while one study reported lower frequency of the 5HTTVNTR 10 repeat allele among TLE patients as compared to controls [15]. Reason of the mentioned discrepancy is not clear at the present and should be addressed in future studies taking into account ethnical influences and/or endophenotype specificities of the case sample, as well as treatment response [9].

Table 1
Genotype and allele distributions of 5HT-related gene polymorphisms in temporal lobe epilepsy (TLE) patients (N = 101) and healthy control subjects (N = 170).

Gene (Polymorphism)			TLE patients		Control subjects		
			N	%	N	%	p[a]
5HTT (5HTTLPR)	Genotype	S/S	14	13.9	17	10.0	0.2498
		S/L	45	44.6	93	54.7	
		L/L	42	41.6	60	35.3	
	Allele	S	73	36.1	127	37.4	0.7834
		L	129	63.9	213	62.6	
5HTT (5HTTVNTR)[b]	Genotype	10/10	21	21.6	24	14.8	0.2344
		10/12	46	47.4	74	45.7	
		12/12	30	30.9	64	39.5	
	Allele	10	88	45.4	122	37.7	0.0961
		12	106	54.6	202	62.3	
MAOA (uVNTR)[b]	Genotype[c]	3/3	7	10.8	15	13.2	0.3416
		3/4	33	50.8	45	39.5	
		4/4	25	38.5	54	47.4	
	Allele	3	60	36.8	93	33.3	0.4698
		4	103	63.2	186	66.7	
5HT-1A (C-1019G)	Genotype	C/C	26	25.7	36	21.2	0.2633
		C/G	51	50.5	78	45.9	
		G/G	24	23.8	56	32.9	
	Allele	C	103	51.0	150	44.1	0.1306
		G	99	49.0	190	55.9	
5HT-1B (G861C)	Genotype	C/C	2	2.0	14	8.2	0.0642
		G/C	35	34.7	65	38.2	
		G/G	64	63.4	91	53.5	
	Allele	C	39	19.3	93	27.4	0.0385
		G	163	80.7	247	72.6	
5HT-2C (cys23ser)	Genotype[c]	ser/ser	2	2.9	4	3.4	0.2863
		ser/cys	11	16.2	28	23.7	
		cys/cys	55	80.9	86	72.9	
	Allele	ser	19	11.2	46	16.0	0.1689
		cys	150	88.8	242	84.0	

[a] p values for differences between groups regarding genotype and allele distributions were calculated by χ^2 and Fisher's Exact Tests, respectively.
[b] Genotypes containing rare alleles of 5HTT VNTR-2 and MAOA-uVNTR polymorphisms were not taken into statistical consideration.
[c] Genotype data refer only to female subjects.

5HT-2C cys23ser polymorphism has been previously shown not to be associated with IGE or alcohol withdrawal seizures [19]. These results, together with the present findings on the lack of association between cys23ser polymorphism and TLE, suggest that cys23ser polymorphism is not involved in the susceptibility to seizure generation. It remains to be determined whether other functional polymorphisms within 5HT-2C gene may play a role in epileptogenesis.

To our knowledge, this is the first study investigating the association of polymorphisms in MAO-A, 5HT-1A and 5HT-1B genes with the epilepsy. While MAO-A and 5HT-1A genetic variants were similarly distributed among TLE patients and control subjects, frequency of 5HT-1B allele 861G was found to be marginally increased in the patient group (Table 1), implicating this allele in the susceptibility to TLE. Huang et al. have found that 861G allele is linked with a fewer 5HT1B receptor sites in human brain, as compared to 861C allele [8]. Reduction of the 5HT1B receptor sites in TLE would fit well into anticonvulsant role of 5HT-1B receptors, as suggested by pharmacological studies on animal models [24,28].

In conclusion, obtained results on 5HTT, MAO-A, 5HT-1A and 5HT-2C suggest that investigated polymorphisms in these genes do not exhibit major effects on the susceptibility to TLE. It should be noted that statistical power of our sample was insufficient for the detection of minor differences (OR < 2), so obtained negative findings do not exclude existence of more subtle effects of these polymorphisms. Observed modest association between G861C polymorphism and TLE seems suggestive and encourages further investigation of the 5HT-1B gene in epilepsy. Because of the possibility of both, false negatives (due to power reasons) or positives (due to multiple comparison bias), presented results should be taken as preliminary until confirmed on larger populations.

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