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Association Analysis of the Functional *MAOA*Gene Promoter and *MAOB* Gene Intron 13 Polymorphisms in Tension Type Headache Patients

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Abstract

Background. Monoamine oxidase (MAO) enzymes play an important role in the etiology of many neurological diseases. Tension type headache (TTH) treatments contain inhibitors for selective re-uptake of serotonin and monoamine oxidase inhibitors. MAO (EC 1.4.3.4) has two isoenzymes known as MAOA and MAOB. A promoter polymorphism of a variable number of tandem repeats (VNTR) in the *MAOA* gene seems to affect MAOA transcriptional activity *in vitro*. Also, G/A polymorphism in intron 13 (rs1799836) of the *MAOB* gene have been previously found to be associated with the variability of MAOB enzyme activity.

Objectives. The aim of our study was to investigate a possible association of monoamine oxidase (*MAOA* and *MAOB*) gene polymorphisms in tension type headache.

Material and Methods. MAO gene polymorphisms were examined in a group of 120 TTH patients and in another 168 unrelated healthy volunteers (control group). MAOA promoter and MAOB intron 13 polymorphisms were genotyped using PCR-based methods.

Results. An overall comparison between the genotype of MAOA and MAOB genes and allele frequencies of the patients and the control group did not reveal any statistically significant difference between the patients and the control group (p = 0.162).

Conclusions. Factors like estrogen dosage, the limited number of male patients and other genes' neurotransmitters involved in the etiology of TTH could be responsible for our non-significant results (Adv Clin Exp Med 2014, 23, 6, 901–906).

Key words: MAOA, MAOB, TTH, PCR-RFLP.

The International Headache Society (IHS) introduced the classification "tension type headache" (TTH) in 1988 and a revised version in 2004. The IHS sub-classifies the diagnosis of TTH into an episodic and chronic form. TTH occurring at least 10 times within a 15 day period is classified as episodic (ETTH). The chronic TTH (CTTH) form must cover additional diagnostic criteria and other defining characteristics according to the IHS classification. These characteristics involve muscle tenderness, physical activity nausea, pain, photo-

phobia and a history of physical and neurological examinations [1–2].

TTH treatment features medication with inhibitors for selective re-uptake of serotonin and monoamine oxidase inhibitors. MAO has 2 isoenzymes known as MAOA (Genbank Acc. No: BC008064.1, Gene ID: 4128, Xp11.3) and MAOB (Genbank Acc. No: M69177, Gene ID: 4129, Xp21-p11). The genes encoding for *MAOA* and *MAOB* are mapped on chromosome Xp11.23. The structures of MAOA and MAOB enzymes are similar

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(70%), but their functions are different. *MAOA* and *MAOB* genes contain 60 kb and 15 exons [3–4].

Two monoamine oxidase (EC 1.4.3.4) isoenzymes MAOA and MAOB are expressed in the outer mitochondrial membrane. Serotonin, dopamine and norepinephrine are substrates preferred by MAOA while phenylethylamine is processed by MAOB. Decreased levels in MAOA activity and *MAOA* gene mutations have been mentioned in connection with criminal, violent or impulsive behavior. [5–7]. MAO enzymes play an important role in the etiology of several neurological diseases. For this reason, the functional polymorphisms of MAO genes are important as it may guide us to the diagnosis and treatment of illnesses which are related to neurotransmitters [8–11].

Furthermore, MAOA catalyzes the oxidative deamination of several biogenic amines, including key neurotransmitters such as serotonin, norepinephrine and dopamine. MAOA is also strongly implicated in the regulation of neurotransmission by cathecolamine and indolamine. Dopamine is metabolized through oxidative deamination by MAOA to form 3,4-dihydroxyphenyl-acetaldehyde (DOPAL) [12]. The activity levels of the MAOA enzyme expose a high range of difference (over 50-fold) in a population [13]. Several lines of evidence indicate that monoamine oxidase, and in particular MAOA, plays an important role in human behavior and physiology [3]. The MAOA gene promoter region contains a 30 bp Variable Number of Tandem Repeat (VNTR) polymorphism. The location of this polymorphism is defined 1.2 kb upstream in the coding sequences of MAOA [11]. Alleles with 3 or 5 copies of the repeat sequence have lesser transcription while a more efficient transcription (2-10 times) is reported for other alleles with 3.5 or 4 copies of repeat [3]. This polymorphism is a strong candidate for association studies of psychiatric disorders and related vulnerability traits [14].

MAOB is an enzyme involved in the metabolism of dopamine, benzylamine, phentylamine, tyramine and tryptamine. The MAOB gene has a single nucleotide polymorphism (SNP) in intron 13, which is based on GA transition (rs1799836) [8]. The G/A polymorphism in intron 13 of the MAOB gene have been previously reported to be related to the variability of MAOB enzyme activity. Altered levels of MAOB have previously been associated with a number of psychiatric and neurological conditions [15-16]. The other studies have suggested that MAOB is potentially relevant to Parkinson's disease because of its role in catabolizing dopamine, with the resultant production of reactive oxidative free radicals [10]. The aim of this study is to examine the relationship between

the G/A polymorphism in intron 13 and TTH patients. In this context, we aimed to assess a possible significance of *MAOA* and *MAOB* genes polymorphism in tension type headache. This study will also be addressing the polymorphic patterns of MAOA and MAOB, both in TTH patients and the healthy population in our country.

Material and Methods

168 healthy volunteers formed our control group and 120 individuals with a TTH diagnosis represent the patients group. The TTH group was subdivided to 2 other groups as episodic TTH (n = 66) and chronic TTH (n = 54) based on clinical diagnostic criteria (IHS-ICHD 2 edition).

There were 106 (86.8%) females and 14 (13.2%) males in the TTH group with mean ages of 37.6 ± 10.6 years, and 100 (59.6%) females and 68 (40.4%) males in the healthy control group with mean ages of 32.9 \pm 18.9 years. The TTH patients and healthy controls were unrelated. TTH patients and healthy controls were nearly age and sex matched. Patients with migraine having any lesion in their brain computed tomography and patients with clear psychiatric disturbance were excluded from the study. Additionally, patients with a history of cervical and cranial trauma were also excluded. Venous blood withdrawal was completed with sampling in ethylene diamine tetra acetic acid (EDTA) containing tubes. The salting out procedure was used for DNA extraction [17].

MAOA gene (Gene ID: 4128) alleles were determined using a Polymerase Chain Reaction (PCR) method [3]. *MAOB* gene (Gen ID: 4129) rs1799836 polymorphism was determined by using the PCR and Restriction Fragment Length Polymorphism (RFLP) methods [10]. The genotypes of *MAOA* and *MAOB* genes were determined by 3% agarose gel electrophoresis containing 0.5 μg/mL ethidium bromide.

A 100 bp DNA Ladder (Fermentas, Vilnius, Lithuania) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat, Marne La Vallée, France). The summarized PCR-RFLP conditions and band sizes are shown in Table 1. The genotype determinations were duplicated in independent experiments and all inconclusive samples were re-analyzed.

Statistical Analysis

The MAOA alleles were divided into 2 groups. Group 1 represents low activity (3-repeat) and group 2 represents high activity (one of 3.5, 4 or 5

Gene	Poly- morphism	RE	Primers Sequence	Ann. temp (°C)	Fragment size (bp)
MAOA	VNTR	-	F 5'-CAGCGCCCAGGCTGCTCCAGAAAC- 3'	65	Allele 1; 221 bp (3 repeats)
					Allele 2; 233 bp (3 and a half repeats)
			R 5'- GGT TCGGGACCTGGGCAGTTGTGC- 3'		Allele 3; 251 bp (4 repeats)
					Allele 4; 281 bp (5 repeats)
MAOB	rs1799836	NmuCI	F 5'- GGAACCTCTTATACCACAGG -3'	54	GG :232 bp
					GA: 232,146,86 bp
			R- 5' GACTGCCAGATT TCATCCTC 3'	1	AA:146/86 bp

Table 1. Primers and Restriction Enzymes (RE) used for MAOA and MAOB genes polymorphisms

repeat units) as described previously [18]. MAOB alleles were also divided into 2 groups: allele G and allele A, as described previously [8]. The data is presented as mean ± standard error for continuous data or frequencies and percentages for categorical data. Chi-square or Fisher exact tests were used to compare proportions of patients for categorical data among groups. Analysis of variance or t-tests were used to compare the continuous variables among groups. We used odds ratio (OR) as a measure of the association of the genetic polymorphisms in MAOA and MAOB with TTH. OR

values were calculated from genotype frequency data. The SPSS 11.5 for Windows program was used for statistical analysis. A p value < 0.05 was considered to be statistically significant.

Results

The visualized samples of polymorphism analyses for MAOA and MAOB genes are presented in Fig.1 and 2, respectively. The participants in both groups were similar in mean age (p = 0.162).

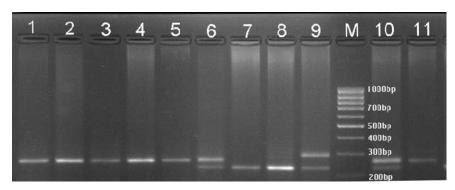


Fig. 1. *MAOA* gene VNTR polymorphism was visualised under UV light using a gel electrophoresis visualising system, samples 1–5 and 11 – 3-3 genotype; samples 6 and 10 – 3-1 genotype; samples 7 and 8 – 1-1 genotype, sample 9 – 1-4 genotype

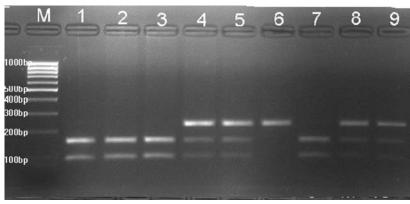


Fig. 2. *MAOB* gene intron 13 polymorphism was visualised under UV light using a gel electrophoresis visualizing system, samples 1–3 and 7 – AA genotype; samples 4, 5, 8, 9 – GA genotype; samples 6 – GG genotype

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MAOA and *MAOB* genes are located on the X chromosome and analyses were performed separately for male and female subjects.

Results for MAOA gene promoter 30 bp VNTR polymorphism revealed that the genotypes were similar in TTH patients and the control group for female (p > 0.05) as well as male subjects (p > 0.05). There was no relationship between episodic TTH, chronic TTH and the control group between females (p > 0.05) and males (p > 0.05). Table 2 represents all of the MAOA genotype results for VNTR polymorphism in our group. Allele

frequencies for *MAOA* gene VNTR polymorphisms are shown in Table 3.

The results of MAOB gene intron 13 polymorphism genotypes were similar in TTH patients and the control group in females and males (p > 0.05). There was also no difference between episodic TTH, chronic TTH patients and the control group between females and males (p > 0.05). The genotypes of MAOB gene intron 13 polymorphisms are shown in Table 2. Table 3 demonstrates the frequencies of G and A alleles of MAOB gene intron 13 polymorphisms in TTH patients and controls.

Table 2. Genotypes of MAOA and MAOB genes in female patients and control groups

Females (XX) Genotypes		Controls (n = 100)		Episodic TTH (n = 58)		Chronic TTH (n = 48)		TTH (n = 106)
		n	%	n	%	n	%	X ² p value
MAOA VNTR	1-1	19	19	11	19	5	10.4	0.657
	1-3	37	37	21	36.2	14	29.2	
	3-3	40	40	22	37.9	23	47.9	
	3-4	2	2	4	6.9	2	4.2	
	1-4	1	1	_	_	3	6.3	
	4-4	1	1	_	-	1	2	
MAOB Intron 13	GG	11	10.9	8	13.8	11	22.9	0.363
	GA	58	57.4	31	53.4	25	52.1	
	AA	31	31.7	19	32.8	12	25	

Table 3. Alleles of MAOA and MAOB genes polymorphisms in patients and control groups

MAOA and MAOB Alleles		Controls (n = 168)		Episodic TTH (n = 66)		Chronic TTH (n = 54)		X ² p value	OR (95% CI)	
		n	%	n	%	n	%		episodic TTH	chronic TTH
MAOA VNTR	1	76	37.9	43	37.1	27	28.1	0.189	reference	reference
Females (XX) Alleles	4	4	2	4	3.4	-	-		4.926 (1.336–18.158)	1.767 (0.421–7.426)
	3	119	60.1	69	59.5	62	64.4		1.467 (0.858–2.506)	1.025 (0.636–1.652)
MAOA VNTR	1	22	32.4	5	62.5	2	33.3	0.21	reference	reference
Males (XY) Alleles	4	-	-	_	-	_	_		_	_
	3	46	67.6	-	_	4	66.7		0.287 (0.063–1.311)	0.957 (0.163–0. 5.626)
MAOB Intron 13 Females (XX)	G	80	39.6	47	40.5	47	49	0.845	1.039 (0.652–1.655)	1.039 (0.652–1.655)
Alleles	A	122	60.4	69	59.5	49	51		reference	reference
MAOB Intron 13 Males (XY)	G	6	75	2	25	3	50	0.288	0.889 (0.167–4.72)	0.296 (0.056–1.573)
Alleles	A	3	50	3	50	6	75		reference	reference

Discussion

The recent definition of MAOA and MAOB gene polymorphisms lead the studies to be focused on the involvement of these polymorphisms in different neurological and psychiatric disorders [19-20]. Related studies have shown that MAOA gene mutations could be associated with borderline mental retardation and abnormal behavior, including increased impulsive behavior [21]. The association between MAOA promoter region polymorphism and migraine was also investigated, but no relation was found [22]. Kelada et al. suggested that the G allele of MAOB had approximately two times the increased risk for PD [23]. Lung F. et al. speculated that MAOA activity might increase in homozygous/hemizygous subjects for the MAOA 4R allele which might lead to abnormal serotonin metabolism in the central nervous system. Their study provided data for an enhanced vulnerability to suicide in depressed males with major depressive disorder, associated with the MAOA 4R allele [24]. In a study by Filic V et al., no association was detected in migraine patients with and without aura [22]. Another study revealed that plasma 5-HT levels in migraine patients without aura are lower

when compared to controls, based on the higher G allele and G/G genotype of T941G polymorphisms of MAOA in migraine patients [25]. Our results failed to show a relationship between MAO enzymes gene polymorphisms and TTH. A similar genetic association was also investigated in patients with chronic daily headache and migraine. Beside other genes studied in that study, MAOA gene polymorphism was also found to be unrelated to headache in the subgroups analyzed [26]. Scientific data provides a strong association between MAO gene polymorphisms and several neurological/psychiatric disorders. Although there is a lack of evidence for a relationship between VNTR polymorphism of the MAOA gene and intron 13 G/A polymorphism of the MAOB gene and TTH, catecholamine metabolism related pathways remain worthy to be investigated in the future to clarify a possible connection.

In conclusion, the authors have not found any association between tension type headache patients and MAO (MAOA, MAOB) genes polymorphism. Further investigations are warranted in larger populations with other susceptible neurotransmitter genes that might be associated with tension type headache.

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