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Monoamine oxidases A and B gene polymorphisms in migraine patients

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Abstract

Abnormal cortical activity and brainstem functioning are considered the possible etiopathogenetic factors of migraine. Monoamine oxidase A and B (MAO-A and -B) regulate the levels of monoamine neurotransmitters, so changes in their activity could participate in migraine pathogenesis. We have investigated the possible association of MAO-A and -B alleles and haplotypes with two common types of migraine, i.e. migraine without aura (MO) and migraine with aura (MA), on the sample of 110 migraineours (80 MO and 30 MA) and 150 controls. MAO-A promoter and MAO-B intron 13 polymorphisms were genotyped by the PCR-based methods. In addition, we have reevaluated the reported association between MAO-B intron 13 polymorphism and platelet MAO-B activity. The platelet MAO-B activity was determined fluorimetrically using kynuramine as a substrate. We have found a tendency toward association of the shorter variant of MAO-A gene promoter with migraine without aura in male subjects. Regarding investigated MAO-B polymorphism, no association with migraine or with platelet MAO-B activity was found. The suggestive association of the variant in MAO-A gene with migraine is considered worthy of independent replication. On the other hand, further studies on MAO-B polymorphism in migraine do not seem promising.

Keywords: Migraine; Monoamine oxidase; Polymorphism; Serotonin; Noradrenaline; Platelet

1. Introduction

Migraine is a neurovascular disease that affects about 15% of the western population [1,2]. Epidemiological studies point that genetic factors play an important role in the etiology of this complex disorder [3]. However, until now, the causative gene has been identified only for a rare familial hemiplegic migraine [4], while for more common types of migraine, i.e. migraine without aura (MO) and migraine with aura (MA), a number of association studies still have not yielded fruitful results [5,6].

One way by which genetic factors could affect the migraine etiopathogenesis is by the control of the metabolism of monoaminergic neurotransmitters. Namely, according to the current theories, the primary causes of migraine pain could be abnormal cortical activity and brainstem nuclei dysfunction [7], with foci in dorsal raphe nuclei and locus coeruleus [8]. Sensory cortices are under the modulation of noradrenergic, cholinergic and serotonergic inputs from brainstem nuclei [9], so it is possible that altered neurotransmitter, especially serotonin and noradrenaline, levels, play a certain role in the migraine etiopathogenesis.

Isoenzymes monoamine oxidases A and B (MAO-A and -B) catalyze oxidative deamination, an essential step in the catabolism of monoamine neurotransmitters and thus participate in functional regulation [10,11]. Genes encoding human MAO-A and -B are located on the short arm of the chromosome X [12,13]. They are arranged in tail-to-tail configuration [14] and both contain common polymorphisms. Upstream variable number of tandem repeats polymorphism in MAO-A gene (MAOA-uVNTR) is located in the promoter region and contains a 30 bp long

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repeated sequence [15]. Several allelic variants (with 2, 3, 3.5, 4, 4.5, 5 and 6 repeats) have been identified, although variants with 3 and 4 repeats constituted more than 97% of the alleles in all reported control samples [15–19]. Expression studies pointed to the functional relevance of MAOA-uVNTR polymorphism by showing that 4-repeats allele was linked to more efficient transcription than 3-repeats allele [15,20]; this relation, however, could not be demonstrated in the postmortem samples of the human brain [21]. MAO-B gene contains A/G dimorphism in intron 13, located 36 bp upstream from the intron 13–exon 14 boundary [22,23]. Initial study of Garpenstrand et al. [24] reported that male individuals with A-allele show significantly lower MAO-B activity in platelets than individuals with G-allele, while the subsequent study on the brain MAO-B activity reported opposite effects of this polymorphism [21]. Association of MAO-A and -B polymorphisms with various neuropsychiatric disorders has been investigated, with results being inconclusive so far [25,26].

Our previous work on the migraine was focused, by the use of platelet model, on the MAO activity [27]. The present study investigates the association of migraine with polymorphisms in MAO-A and -B genes. In addition, it also reevaluates, on a larger sample and different ethnicity, the reported correlation between MAO-B polymorphism and the platelet MAO activity [24].

2. Materials and methods

2.1. Subjects

Blood samples were collected from the clinic outpatients of Department of Neurology of Sveti Duh General Hospital, Zagreb. The study included 110 patients suffering from migraine (age 35.7±14.0 years): 80 from migraine without aura (56 women and 24 men) and 30 from migraine with aura (24 women and 6 men). The diagnoses were made according to the International Headache Society criteria [28]. The study was approved by the Ethics committee of the Medical faculty, University of Zagreb. The control group consisted of 150 healthy blood donors (age 41.4±11.2 years), 100 women and 50 men, without personal or family history of headache or other neurological or psychiatric disorders, as reported in a written interview. The correlation between MAO-B gene polymorphism and platelet MAO-B activity was studied on a separate group of 70 healthy male blood donors, nonsmokers, age 32.4±9.4 years. All participants signed an informed consent before entering the study.

2.2. Genotyping

Genomic DNA was isolated from the peripheral blood using standard phenol chloroform extraction. For MAOA-uVNTR polymorphism 100 ng DNA was amplified by polymerase chain reaction (PCR) in a mixture containing 0.2 μM primers, 200 μM each dNTP, 1.5 mM $MgCl_2$ and 0.01 U μL^{-1} Taq DNA polymerase (Promega) in a final volume of 20 μL. The primer sequences were taken from the previous study [15]. Cycle conditions were as follows: 2 min at 95 °C, 35 cycles (30 s at 95 °C, 30 s at 61 °C and 40 s at 72 °C), and 7 min at 72 °C. Genotyping was performed according to the length of PCR products (276, 306, 321, 336 and 351 bp for 2, 3, 3.5, 4 and 4.5 repeats, respectively) separated on the 10% polyacrylamide gel. MAO-B dimorphism was genotyped by allele-specific oligonucleotide PCR (ASO-PCR), using published primer sequences [22]. The reaction mixture of 15 μL contained 75 ng of genomic DNA, 0.2 μM primers, 50 μM each dNTP, 1.5 mM $MgCl_2$ and 0.003 U μL^{-1} Ampli Taq DNA Gold polymerase (Perkin Elmer). The cycling conditions were as follows: 95 °C for 10 min (hot start), 35 cycles (30 s at 95 °C, 30 s at 61 °C and 40 s at 72 °C), and 72 °C for 5 min. The presence or the absence of 663 bp long PCR product was checked after electrophoresis on 1.6% agarose gel stained with ethidium bromide.

2.3. Measurement of platelet MAO-B activity

Preparation of platelet-rich plasma (PRP) was done by the method described previously [27]. Enzyme velocity was expressed as nanomoles 4-HQ per 10^8 platelets per 60 min and K_M as μM concentration of kynuramine.

Table 1
Allele and genotype frequencies of MAOA-uVNTR polymorphism among migraine patients and controls

Sample (*N*)		Allele, *N* (%)		*p*	Genotype, *N* (%)			*p*
		4	3		4/4	4/3	3/3	
Females	controls (96)	127 (66.1)	65 (33.9)		46 (47.9)	35 (36.5)	15 (15.6)	
	MO (55)	67 (60.9)	43 (39.1)	0.3840	18 (32.7)	31 (56.4)	6 (10.9)	0.0598
	MA (22)	33 (75.0)	11 (25.0)	0.2878	12 (54.6)	9 (40.9)	1 (4.5)	0.3915
Males	controls (49)	34 (69.4)	15 (30.6)					
	MO (23)	10 (43.5)	13 (56.5)	0.0423*				
	MA (6)	3 (50.0)	3 (50.0)					

MO=migraine without aura; MA=migraine with aura.