

Effect of monoamine oxidase B A644G variant on nicotine dependence and/or schizophrenia risk

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Abstract

Objectives: Schizophrenia (Sch) is a severe and chronic mental illness. Smoking prevalence is higher in patients with Sch than general population. We aimed to investigate the effects of *MAOB* gene A644G variant on nicotine dependence (ND) and Sch+ND risk in Turkish population and to evaluate by bioinformatic analysis. **Methods:** Present study included 161 individuals with ND, 223 patients with Sch+ND, and 96 non-smoker controls. *MAOB* A644G variant was analyzed using PCR-RFLP method. As the *MAOB* gene is located on the X chromosome, each gender was analysed separately. **Results:** The total distributions of AA, AG and GG genotypes of *MAOB* gene A644G were 44.7%, 22.4% and 32.9% in the ND group, 45.3%, 25.1% and 29.6% in the Sch+ND group and, 44.8, 22.9% and 32.3% in non-smoker controls. No significant differences were observed between groups for the *MAOB* A644G genotype and allele frequencies when female group compared to male group ($p > 0.05$). Examination of disease associations of SNPs from each miRNA gene region in GWAS databases yielded results for aging, bipolar disorder, autoimmune, and neurological diseases. **Discussion:** Our results indicate that the *MAOB* gene A644G variant is not associated with ND and/or Sch susceptibility in the Turkish population.

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Introduction

Schizophrenia (Sch) is a chronic and severe mental disorder with an elusive psychopathology. This disease can be extremely devastating and interferes with cognition, emotion, perception, and other behavioral aspects¹. Inheritance has been found in up to 80% of the cases², however, the exact etiology of this disorder is still unclear. Many researchers have proposed that dysregulated dopaminergic neurotransmission is involved in the pathogenesis³. Dopamine acts as a neurotransmitter by binding to its specific receptors on the postsynaptic membrane and autoreceptors on the presynaptic membrane.

Tobacco use is the only most preventable cause of death, disability and illness in the World. Nicotine, the major active component of cigarettes that plays a role in physical dependence, influences nicotine receptors in the central nervous system and results in the release of neurotransmitters (including dopamine). Nicotine activates dopaminergic neurons in the mesolimbic reward system and induces dopamine release⁴. The prevalence of smoking is much higher among people with psychotic disorders compared to the general population. Besides, among those suffering from Sch, the nicotine dependence (ND) occurs more frequently than both the general population and those with other psychiatric diseases⁵.

Genes that play a role in neurotransmitter metabolism pathways have been widely examined in studies of psychiatric disorders. Monoamine oxidase (*MAO*; E.C. 1.4.3.4) is a flavin-adenine-dinucleotide (FAD)-containing enzyme that plays a role in the metabolic breakdown of several biogenic amines⁶. It is found on the outer membrane of mitochondria in most cell types. Two isoenzymes (*MAOA* and *MAOB*) of *MAO* are present. In humans, *MAOA* ideally oxidizes serotonin and noradrenaline, while *MAOB* oxidizes dopamine⁷. *MAOA* and *MAOB* genes are sequenced tail to tail on the Xp11.23 chromosomal region, and both genes have 15 homologous exons (1). A644G single nucleotide polymorphism (SNP) (rs1799836) in intron 13 of the *MAOB* is known functional polymorphism⁸.

Therefore, we conducted a case-control study in a Turkish population, to assess the impact of the *MAOB* A644G variant on risk ND and/or Sch+ND. As the *MAOB* gene is located on the X chromosome, each gender was analyzed separately.

Methods

Study population

The study sample included 161 subjects with ND (87 male and 74 female; mean age: 45.93 years), 223 subjects with Sch+ND (105 male and 118 female mean age: 46.78 years), and 96 unrelated healthy controls (41 male and 55 female; mean age: 44.91 years). The ethnicity of all participants was Turkish. The patients with Sch were recruited from the Department of Psychiatry Clinic, Bakirkoy Mental Health Research and Training Hospital, Istanbul, Turkey and diagnosed according to the Diagnostic and Statistical Manual of Mental Disorders, 4th ed. (DSM-IV)⁹. ND group was selected from the Department of Chest Diseases, Yedikule Hospital for Chest Diseases and Thoracic Surgery Training and Research Hospital, Istanbul, Turkey. ND group consisted of active smokers. These subjects were defined as those who had previously smoked more than one cigarette per day but had quit smoking for more than one year. The degree of ND was evaluated by the scores on Heaviness of Smoking Index (HSI) and the Fagerström Test for Nicotine Dependence (FTND)¹⁰. Control group was recruited from “non-smokers” were defined as those who had smoked less than one cigarette per day for no more than 1 year during their lifetime. Before enrollment, signed informed consent was obtained from every participant or their guardians if the participant was a minor or could not provide consent. The study protocols were performed according to the principles of the Declaration of Helsinki. This study was approved by the Ethics Committees of the Istanbul University, Istanbul Medical Faculty.



Genotyping

Blood samples were collected from all participants using EDTA tubes and DNA was extracted from whole blood using salting out method¹¹. DNA samples were stored at -20 °C until genotype analysis. The MAOB A644G variant was analyzed by polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) assays¹². The segment was replicated by using the following forward and reverse PCR primers: 5-GGAACCTCTTATACCACAGG-3 and 5-GACTGCCAGATTCATCCTC-3. Then the DNA products were digested with the restriction enzyme Tsp 45I. MAOB allele 1 (containing A and therefore the Tsp 45I restriction site) was detected as two bands of 146 and 86 bp, whereas allele 2 (containing G and no Tsp 45I restriction site) was detected as a single 232-bp band. Since the MAOB gene is found on the X chromosome, i.e. males carry only one allele for MAOB.

Bioinformatic analysis

We examined the regulation of expression of MAOB (microRNAs (miRNAs); co-regulated gene sets), and disease associations of MAOB sequence variants. Gene co-expression networks can be used to associate genes of unknown function with biological processes, to prioritize candidate disease genes. We used the CO-Regulation Database (CORD) for co-expression analysis, GSEA/MSigDB for gene enrichment analysis, TargetScan for miRNAs targeting MAOB and GRASP as GWAS Database for disease associations of MAOB and miRNA region SNPs. Also, we used¹³ for enhancers effecting MAOB expression and other genes.

Statistical analysis

The genotype distribution and allele frequency of the MAOB A644G variant in the control and patient groups were compared using Chi-squared tests. The Hardy-Weinberg equilibrium (HWE) was calculated using the de Finetti program (Online HWE and

Association Testing-Institut für Humangenetik, Munich, Germany). Odds ratio (OR) and 95% confidence intervals (CIs) were estimated using the binary logistic regression method. *p* values less than 0.05 were considered statistically significant.

Results

In the present study, a total of 480 subjects, including 161 subjects with ND, 96 non-smoker control, and 223 patients with Sch+ND were genotyped for the A644G variant of MAOB gene. The genotype and allele frequencies of the MAOB A644G variant in the studied groups was shown in table 1. There were 74 (45.9%) female and 87 (54.1%) male in the ND group and 55 (57.3%) female and 41 (42.7%) male in the control group (non-smoker) and 118 (52.9%) female and 105 (47.1%) male in Sch+ND group. The prevalence of total genotypes of AA/A, AG, and G/GG profiles for MAOB A644G variant were 44.7%, 22.4% and 32.9% respectively in the ND group, 44.8%, 22.9%, and 32.3% in non-smoker group, and 45.3%, 25.1% and 29.6% in the Sch+ND group.

The statistically significant relationship was not found between groups for the MAOB A644G genotype and allele frequencies (*p* > 0.05).

Discussion

Sch has been considered as a complex neurodevelopmental disorder. The brain's monoamine (dopamine, serotonin and norepinephrine) systems are involved in normal behavior and pathology within these circuits is suggested to underlie several neurological and psychiatric conditions. Dopamine neurons in the midbrain manifest various unique activity states that have implications for the function of the dopamine system. A dopamine hypothesis of Sch proposes a final common pathway of presynaptic striatal hyperdopaminergia resulted by an interaction between numerous environmental and genetic risk factors influencing brain function that lead to negative and cognitive symptoms⁵.

Table 1. Genotype and allele frequencies of MAOB A644G variant in ND, control and Sch+ND groups

MAOB A644G	Genotypes	ND group	Control group	Sch+ND group	OR	%95 CI*	<i>p</i>
		n (%)	n (%)	n (%)			
Females	A/A	24 (32.4)	17 (30.9)	41 (34.7)	1.030	0.751-1.413	0.854 ^{p1}
					0.887	0.550-1.429	0.753 ^{p2}
					0.937	0.638-1.377	0.862 ^{p3}
	A/G	36 (48.7)	22 (40.0)	56 (47.5)	1.160	0.862-1.559	0.425 ^{p1}
					0.812	0.518-1.272	0.451 ^{p2}
					1.031	0.720-1.472	0.990 ^{p3}
G/G	14 (18.9)	16 (29.1)	21 (17.8)	0.770	0.508-1.165	0.208 ^{p1}	
				1.508	0.957-2.376	0.136 ^{p2}	
				1.047	0.666-1.645	0.849 ^{p3}	
Males	A	48 (55.2)	26 (63.4)	60 (57.1)	0.898	0.709-1.137	0.491 ^{p1}
	G	39 (44.8)	15 (36.6)	45 (42.9)	1.209	0.702-2.082	0.613 ^{p2}
					0.957	0.700-1.308	0.898 ^{p3}
Total	A/A+A*	72 (44.7)	43 (44.8)	101 (45.3)	0.858	0.718-1.025	0.117 ^{p1}
					0.986	0.704-1.380	0.994 ^{p2}
					0.939	0.743-1.189	0.679 ^{p3}
	A/G	36 (22.4)	22 (22.9)	56 (25.1)	0.998	0.786-1.241	0.917 ^{p1}
					0.918	0.614-1.373	0.782 ^{p2}
					0.914	0.685-1.218	0.616 ^{p3}
G/G+G*	53 (32.9)	31 (32.3)	66 (29.6)	1.011	0.728-1.235	0.915 ^{p1}	
				1.092	0.765-1.557	0.728 ^{p2}	
				1.093	0.853-1.400	0.559 ^{p3}	

ND: Nicotine dependence; Sch: schizophrenia; P¹: ND versus control; P²: ND versus Sch+ND; P³: control versus Sch+ND. * The genotype distribution of MAOB A644G variant were compared as female, male and total (female+male) groups.

Smoking is commonly encountered in Sch cases and it is one of the main causes of early death in this illness. Even though causes for such high frequency of cigarette smoking in patients with Sch remain unclear, dopamine seems to play a crucial role in this correlation. It has been shown that the addictive effects of smoking are not limited to the effects of nicotine, but may also be associated with the high dopamine levels¹⁴. Nicotine elevates dopamine levels in striatum by inducing its release via nicotinic receptors, and diminishing its breakdown by hindering monoamine oxidase type A and B¹⁵. Due to crucial role of dopamine in ND, variants in several dopamine related genes have been suggested and examined for their potent relations with smoking behaviour.

MAO catalyses the oxidative deamination of numerous biogenic amines, and is involved in the regulation of neurotransmitters in the central nervous system, such as modulation of synaptic concentrations of serotonin, dopamine, norepinephrine and other catecholaminergic neurotransmitters. Enhancers affecting MAOB expression and other genes: [EFHC2 (EF-hand domain containing 2) and MAOA are also targeted by the same enhancers affecting MAOB expression]. An important paralog of this gene is MAOA. When 5-fold threshold was used, there were 192 concordantly co-expressed genes. (PHYH, ALDH6A1, RGN, FXYD1, MLXIPL, ISOC1, DDO, GSTM4, SLC6A12, AMACR, CRP, FLJ39378, FLJ21963). Using TargetScan we selected the miRNAs targeting MAOB with a context score of less than -0.30, and determined their other target genes. Altogether, 26 miRNAs were identified as targeting MAOB¹⁶.

Variations in MAO enzyme expression and activity may contribute to genetic susceptibility for becoming a smoker, and could affect the ability of a smoker to quit. MAOB A644G variant leads to a transitional conversion from adenine (A) to guanine (G) 36 bp upstream from the 5'-end of exon 14. It is proposed that, because of its position, this variant alters the stability and/or translation of MAOB mRNA¹⁷. Studies have been previously conducted to investigate the relation with smoking for polymorphisms of dopamine metabolic enzymes. Ito *et al.* found a decreased risk of being current smokers for individuals carrying the 4-repeat allele of the MAOA VNTR polymorphism in the promoter region for women, and the results were the opposite for men¹⁸. Costa-Mallen *et al.* suggested that MAOB G644A was not associated with smoking status in either men or women, however, they reported that there was an interaction between MAOB A644G and the D2 dopamine receptor (DRD2) gene TaqIB variants; they also proposed that subjects carrying MAOB allele A and genotype B12 of DRD2 were 2.50 times more likely to be ever-smokers than the pool of men carrying all other genotype combinations¹⁹. Fowler *et al.* showed that both type A and type B MAO are inhibited in the brains of smokers²⁰. Also, it was found a significant association between the MAOB intron 13 allele and different levels of MAOB enzyme activity in human brain¹⁷. Tiili *et al.* reported that the MAOB intron 3 AG variant G allele was more prevalent in female smokers than non-smokers²¹. Also, it was reported that A allele in MAOB G644A variant reduced the risk of heavy smoking in males²². However, Tochigi *et al.* obtained no evidence for an association between the MAO genes and smoking behavior, including epistatic or additive effects²³.

There are several studies suggest that MAO genes may be implicated in the susceptibility to Sch. Investigated MAOA gene variants mostly haven't been linked with Sc²⁴⁻²⁵. For MAOB A644G variant and Sch, a sex specific association has been found in Spanish population, with a high prevalence of G allele in women diagnosed with Sch compared to healthy women controls²⁶. Although Peitl *et al.* reported that MAOA VNTR variant did not reveal statistically significant findings in overall samples, they found that MAOA VNTR variant allele 3 was statistically more frequent in male patients with Sch²⁷. Bergen *et al.* reported that there was an association between a MAOB microsatellite polymorphism and particular clinical features of patients with Sch²⁸. In studies investigating Sch symptoms, Camarena was found that there were statistical differences between MAOB A644G variant and affective flattening scores in patients with Sch²⁹. But Zammit *et al.* supported

that genotypes of MAOA and MAOB genes are not risk factors for aggressive behavior in Sch³⁰.

In present study, we investigated the possibility that MAOB A644G variant may also be involved in predisposition to ND and ND+Sch. Since the MAOB gene is found on the X chromosome, each gender was usually analyzed for both genders. To the best of our knowledge, this is the first association study of MAOB gene A644G and ND and/or Sch risk in a Turkish population. The our results did not show the significant association between the genotype/allele frequencies of the MAOB A644G variant and ND and/or Sch+ND risk.

The present study, however, bear some limitations. First, the findings may be limited to the Turkish population. Ethnic variations may also play a role in association studies. We could not determine MAOB expression. Another limitation is that Sch subgroups are not specified. Furthermore, the present study has a relatively small population size and may not have the necessary strength. Therefore, larger sample sizes are needed to identify smaller effects, possibly of little clinical significance.

In conclusion, our preliminary findings could indicate the MAOB A644G variant was not associated with ND and/or Sch+ND risk in a Turkish cohort. The complex nature of psychiatric illnesses mandates a multidisciplinary approach involving genetics, neuroscience, psychiatry, and molecular biology in order to achieve a final conclusion. As for now, we suggest that further large-scale studies should be conducted to clarify the role of this gene in ND and Sch pathogenesis to obtain more insight into this hypothesis.

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Informed consent

Written informed consent was obtained from subjects and patients who participated in this study.

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