

Lack of association between MAOA-uVNTR variants and excessive daytime sleepiness

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Abstract Sleep disorders are highly prevalent in the population and have dramatic health, social, and economic impacts. However, their treatments may remain symptomatic due to ignorance of molecular factors which may provide fundamental insights into the neurological bases of sleep. Excessive daytime sleepiness (EDS) is a common complaint encountered in neurological practice with significant effects both on individuals and on society. We aimed to investigate the role of monoamine oxidase A (MAOA) as a candidate gene in EDS. Epworth sleepiness scale (ESS) was applied to 221 subjects who were also genotyped for MAOA upstream variable number of tandem repeats (MAOA-uVNTR). Patient group displayed higher ESS values (mean 12.67) when compared with the control group (mean 6.38). However, MAOA-uVNTR genotypes did not show a significant association with ESS scores neither on women nor on men. Finally, these data suggest further replications in different populations. Moreover, the investigation of some other genes together with MAOA

and/or some possible regulatory molecular mechanisms may offer a more comprehensive approach in the role of genetic factors contributing to EDS.

Keywords Monoamine oxidase A · MAOA-uVNTR · Sleep · Neurogenetics · Excessive daytime sleepiness · Variable number tandem repeat

Introduction

Excessive daytime sleepiness (EDS) is a common complaint encountered in neurological practice. EDS can have various and serious consequences both on individual and society level. On the individual level, the symptoms significantly lower the personal effectiveness at school or work, lead to memory and mood disorders, and also affect ability to gain or maintain employment since patients with EDS may be misperceived as lazy or unmotivated. On the societal level, the negative outcomes of sleepiness are also very important in terms of contributing to motor vehicle accidents, work-related incidents, and deaths [1, 2].

The gene encoding MAOA is located on chromosome Xp11.23 [3] and contains a polymorphism termed as MAOA upstream variable number of tandem repeats (MAOA-uVNTR) located 1.2 kb upstream of the MAOA coding sequences [4]. An optimal length for the regulatory region is assumed since the alleles with 3.5 or 4 copies of the repeat sequence are transcribed 2–10 times more efficiently than those of three or five copies [4, 5].

As being a reasonable candidate in the manifestation of psychological states, we aimed to investigate the role of MAOA-uVNTR alleles in daytime sleepiness. The investigations of MAOA-uVNTR variants on excessive daytime sleepiness are very limited. Previously published two

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studies [6, 7] evaluating the role of MAOA-uVNTR variants in EDS seem to contradict with each other though they used different sleep quality tools [Pittsburgh Sleep Quality Index and Epworth Sleepiness Scale (ESS), respectively]. Our study might be determinative of solving the apparent discrepancy.

Materials and methods

Participants

We recruited a total cohort of 221 subjects in Turkey [patient number: 93 (42.1%); control number: 128 (57.9%)]. The patient group consisted of 52 females (55.9%) and 41 males (44.1%), while the control group consisted of 61 females (47.7%) and 67 males (52.3%). Since obesity is one of the most important contributing factors for daily sleepiness, the incidence of participants over threshold 30 kg/m² which is an indicator of obesity was also recorded. Participants who were experiencing any disorders such as hypo/hypertension, diabetes mellitus, thyroid function disorders, and a drug history of antidepressant use that can affect the sleepiness evaluation were excluded from the study. Demographic features (age, gender), basic anthropometric measurements (height, weight), concurrent comorbidities, drug use, and ESS values of all the participants were recorded. The participants were further described according to their anthropometric measurements and health status (Table 1). Out of 93, 47 patients with complaints of snoring, apnea, night sweating, and ESS ≥ 12 had undergone additional polysomnographic investigation for differential diagnosis of obstructive sleep apnea syndrome (OSAS). All participants donated approximately 5-ml blood samples for further genetic analysis. The study was approved by Medeniyet University Medical Center Institutional Review Board, and all subjects gave written informed consent prior to their participation in the study. Data were collected with the collaboration of both a family physician and a neurologist from Sleep Research Laboratory.

As mentioned at Table 1, health status of both groups was plausible with our inclusion criteria, and there was not any history of antidepressant medications. Obesity and insulin resistance were the most common disorders in both groups. The reported people received metformin therapy for insulin resistance and were under control of an endocrinologist. The other medications were anti-ischemics/anticoagulants for coronary heart disease; proton pump inhibitors for gastrointestinal disorders; antibiotics for upper respiratory tract and urinary tract infections; calcium and vitamin D supplements; and nonsteroid anti-inflammatory drugs for musculoskeletal disorders. None of

the drugs above are reported to have any effect on sleep status. Most of the medications were occasionally used in case of feeling a symptom for instance antibiotics and pain killers.

Measures

Genotyping

Genomic DNA was extracted from 5 ml of blood in accordance with salting-out method [8], and an aliquot of DNA was used for MAOA genotyping with the primers previously published in the literature [9] with some modifications in PCR protocol. Amplification by the polymerase chain reaction (PCR) was performed in a final volume of 25 μ l, which contained ~ 100 ng of DNA, 2 mM MgCl₂, 300 μ M each dNTPs, 0.5 μ M each of two primers, and 2 U of Taq polymerase (Thermo Scientific, USA). PCR was performed on a gradient thermal cycler (Bioer, Gene-Pro, China) with the following cycling conditions: 1 cycle of pre-denaturation at 95 °C for 5 min, 35 cycles of denaturation at 94 °C for 1 min, annealing at 58 °C for 1 min, extension at 72 °C for 1 min 30 s, and a final extension step at 72 °C for 10 min. Polymerase chain reaction products were separated by 3% agarose gel electrophoresis stained with ethidium bromide (EtBr) and visualized on gel documentation system (Uvitech Cambridge, Fire-Reader, UK), and genotypes were evaluated with a molecular weight marker (GeneRuler Low Range DNA Ladder, Thermo Scientific, USA). MAOA promoter uVNTR polymorphism was interpreted on the results of previous study [9] in which PCR product sizes for the 2R, 3R, 3.5R, 4R, and 5R allelic variants were 290, 320, 335, 350, and 380 bp, respectively.

Sleep measurement

Epworth sleepiness scale (ESS) was used to quantify the general level of daytime sleepiness [10]. ESS was designed to measure sleep propensity in a simple, standardized way for the eight situations to give a score for each subject, between 0 and 24.

Statistical data analysis

Of the 233 subjects who completed, the ESS were subjected to MAOA-uVNTR genotyping. Six individuals could not be genotyped and also individuals with infrequent 2-, and 5-alleles were excluded from further statistical analyses owing to their low frequencies (a 2-repeat allele was found in one female, and a 5-repeat allele was found in one male and four females). Thus, our final cohort of 221 subjects (male = 108, female = 113) were

Table 1 Demographic characteristics and additional health status of both groups

	Patient group (<i>n</i> = 93)	Control group (<i>n</i> = 128)	<i>p</i> value
Gender			–
<i>n</i>	52/41	61/67	
Female/male (%)	55.9/44.1	47.7/52.3	
Age (years), mean ± SD	48.22 ± 15.94	42.17 ± 14.89	0.004
Height (cm), mean ± SD	169.3 ± 9.78	165.69 ± 11.82	0.018
Weight (kg), mean ± SD	81.35 ± 21.17	74.92 ± 17.63	0.015
BMI (kg/m ²), mean ± SD	32.87 ± 46.40	28.45 ± 21.99	0.348
BMI >30 kg/m ² , <i>n</i> /%	30/32.2	32/25	0.639
BMI >35 kg/m ² , <i>n</i> /%	10/10.7	9/0.7	0.592
Other disorders (<i>n</i>)	28	37	
COPD	5	0	
Hyperlipidemia	7	0	
Cardiac ischemic disorders	5	1	
IR/obesity	9	16	
URTI	2	4	
Iron deficiency	0	3	
Gastrointestinal or urinary disorders	0	8	
Muskuloskeletal disorders	0	5	
<i>n</i> of participants ESS ≥12	61	15	

COPD chronic obstructive pulmonary disease, IR insulin resistance, URTI upper respiratory tract infection, ESS Epworth Sleepiness Scale

investigated via statistical analysis to investigate the effect of MAOA-uVNTR on ESS scores. Of the study cohort of 221 subjects, 93 individuals (42.1%) were in the patient group, and this consisted of 52 females (55.9%) and 41 males (44.1%). The individuals in the control group consisted of 61 females (47.7%) and 67 males (52.3%) (Table 1). Individuals were grouped for statistical analyses into the following two classes: 3- and 4-repeat male hemizygotes, and female carriers with 3/3, 3/4, and 4/4 genotypes. Given that MAOA is located on the X chromosome, the phenotype of MAOA may differ between males and females. Therefore in this study, all of the analyses were conducted separately for males and females; one-way ANOVA for females and *t* test statistics for males were used for the comparisons of MAOA genotypes with ESS scores. Genotype frequencies were assessed for Hardy–Weinberg equilibrium, only in females, with a χ^2 test.

ESS scores were tested for normal distribution using the Shapiro–Wilk test. Based on the approach in the literature [7], the 75th percentile of the ESS score distribution was used as a cut-off point to determine normal (score ≤13) or high (score ≥14) sleepiness categories in our study cohort instead of a fixed cut-off model. Also a linear regression model was carried out to assess the relationship between ESS scores and MAOA genotypes, and the comparison of MAOA genotype frequencies with daytime sleepiness categories (high, normal) was assessed with logistic

regression analysis. A *p* value <0.05 was considered as statistically significant.

The differences in patient and control groups in terms of ESS scores and the relationship between people with or without drug use in the patient group were investigated with *t* test statistics. *t* test model was also used to assess the relations among sleepiness categories (normal, high) and measures of age, weight, height, and body mass index (BMI).

Results

The mean age, weight, height, and BMI values of the patient group were 48.22 (±15.94), 81.35 (±21.17), 169.3 (±9.78), and 32.87 (±46.4), respectively. The mean age, weight, height, and BMI values for the control group were calculated as 42.17 (±14.89), 74.92 (±17.63), 165.69 (±11.82), and 28.44 (±21.98), respectively.

When MAOA genotype frequencies (three and four repeats) were assessed for females, this polymorphism was found to be in Hardy–Weinberg equilibrium (*p* = 0.153). Frequencies of the 3-repeat and 4-repeat alleles were 0.49 and 0.48, respectively.

ESS scores ranged from 0 to 24 with a mean value of 9.17 (SD 5.38). ESS scores in our cohort had a normal distribution according to the Shapiro–Wilk test (non-

significant p value). ESS scores of the patient group were found higher (mean 12.67) than the control group (mean 6.38). However, ESS scores did not show a difference in terms of drug use in the patient group ($p > 0.05$). ESS scores did not show a statistically significant association with MAOA genotypes neither on women nor on men (Table 2). Regression analysis data also did not display a statistically significant association with the comparisons of MAOA genotypes with ESS scores or daytime sleepiness categories (normal, high) ($p > 0.05$) (Table 3). Distribution of the frequency of ESS score groups (normal, high) according to MAOA genotypes is depicted in Fig. 1.

Discussion

The frequencies of 3- and 4-repeat alleles of MAOA-uVNTR were 49 and 48%, respectively, in our study cohort. As reported before, frequency distributions of 3- and 4-alleles exhibit variations significantly among different ethnic/racial groups [4, 11], and our study is thus important in terms of reflecting the situation in Turkish population.

MAOA has been considered as a reasonable candidate in the manifestation of psychological traits and psychiatric disorders since it regulates monoamine turnover and thus finally affects the levels of norepinephrine, dopamine, and serotonin [5]. Because of its role in driving behavioral manifestations, it has earned the nickname ‘warrior gene.’ MAOA gene has been found to be associated with aggression since transgenic mice with MAOA gene deletion exhibited behavioral alterations [12]. A Dutch family with repeated violent criminal behaviors among male members during several generations was reported to have an abnormality in MAOA gene [13]. The regulation of emotion and cognition in the limbic system may be affected by MAOA. It was found that carriers of the low-activity MAOA polymorphism showed 8% reductions in gray matter volumes in the amygdala, cingulate gyrus,

insula, and hypothalamus compared with the carriers of the high-activity variants [14].

MAOA-uVNTR may have effects on serum lipid levels and individuals with less active alleles may be at increased health risk of developing coronary heart disease with the cumulative effects of other tendencies such as alcoholism, antisocial personality disorder, sleep disorder, increased depressive symptoms, and higher BMI's which are also related to a certain extent of low-activity alleles [5, 15]. Brummett et al. did not find a significant relation between BMI and MAOA-uVNTR alleles [5]. In our study, BMI was not related to MAOA-uVNTR alleles and normal/high sleepiness categories either. On the other hand, we can not rule out the fact these findings can show changes according to the population cohorts and also be shaped by some other factors.

MAOA genotype also displays sex-dependent properties, and this was emphasized in several studies in the literature [16–23].

Daytime sleepiness tests in clinical practice include ESS, Multiple Sleep Latency Test (MSLT), and Maintenance Wakefulness Test (MWT). MSLT is used in the evaluation of daytime sleepiness in the absence of environmental stimuli while MWT is used for the evaluation of patients after therapy. MSLT is used for the suspect of narcolepsy for early-onset REM diagnosis, whereas other two tests can be used in normal individuals being searched for daytime sleepiness [24, 25]. The reliability of ESS is also close to MSLT-SL [26]. The individuals in our study were evaluated according to the sleepiness scales without considering sleep latency and also OSAS, and narcolepsy patients included had been evaluated with polysomnography (PSG) and MSLT to overcome study limitations. Epworth sleepiness scale is a screening test, and the definition and evaluation are made by the patient. Score of ESS ≥ 12 or the presence of other OSAS symptoms is a clear indication of the necessity of further investigations. Unfortunately, the authors did not collect any data on sleep-wake schedule of the participants. The participants' self reports on being day long sleepy were taken into consideration. This is a limitation of our study.

The results of our study demonstrated that MAOA-uVNTR alleles do not confer tendency to EDS. Further larger-scale researches in different populations can help us to strengthen this plausible hypothesis. Moreover, one approach in other studies may be the investigation of some other genes involved in the serotonergic pathway together with MAOA and/or the regulatory mechanisms that may have effects on MAOA activity such as methylation. The relatively high variability of EDS in pediatric OSA was shown to be related with underlying TNF- α gene polymorphisms, particularly –308G [27]. In another study, an association was found between OSAS and serotonin (5-

Table 2 Lack of association between Epworth Sleepiness Scale (ESS) scores and MAOA genotypes according to parametric tests

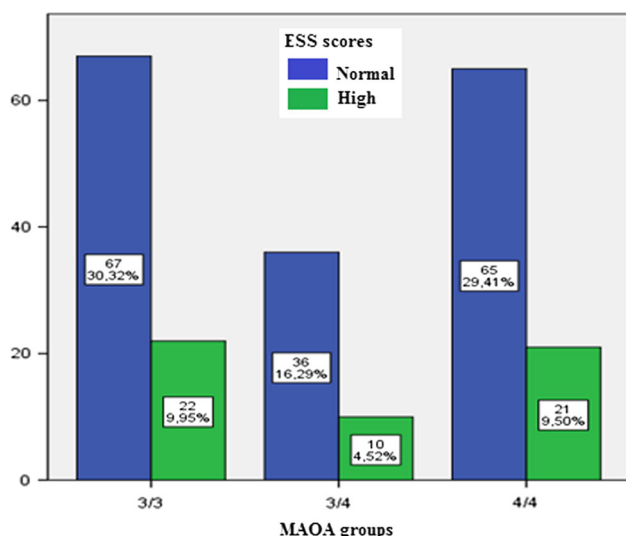
	MAOA groups	ESS score mean-SD	p
Male	3/3	12.48	0.907 ^a
	4/4	12.63	
Female	3/3	13.36	0.934 ^b
	3/4	12.89	
	4/4	12.67	

^a p value for comparison of scores between genotype groups for male, using a t test statistics

^b p value for comparison of scores between genotype groups for female, using an ANOVA

Table 3 Lack of association of Epworth Sleepiness Scale (ESS) scores and MAOA genotypes according to regression analysis

MAOA groups	N (%)	Mean ESS scores (SD)	MAOA groups	Normal sleepiness n (%)	High sleepiness n (%)
3/3	89 (40.3)	9.32 (5.48)	3/3	67 (39.9)	22 (41.5)
3/4	46 (20.8)	8.27 (5.38)	3/4	36 (21.4)	10 (18.9)
4/4	86 (38.9)	9.23 (5.37)	4/4	65 (38.7)	21 (39.6)
Total	221 (100)		Total	168 (100)	53 (100)

^a *p* value: 0.231^a^b *p* value: 0.112^b^a *p* value for comparison of scores between genotype groups, using a linear regression model^b *p* value for comparison of MAOA frequencies between normal and high sleepiness groups, using a logistic regression**Fig. 1** The frequency of ESS score groups according to MAOA genotypes

HT) polymorphism –1438 G/A [28]. A SNP (rs2859998) within the UBXN2B, an adaptor protein required for golgi and endoplasmic reticulum biogenesis, was strongly associated with the age at onset of EDS [29]. Whether EDS symptoms would correlate with other genes involved in sleep regulation must further be investigated.

The prevalence of excessive sleepiness was clearly related to obstructive sleep apnea (OSA) severity [30]. Excessive daytime sleepiness is the most common symptom of OSA. In patients with OSA, approximately 23% of women and 16% of men experience excessive daytime sleepiness. Besides, in some studies, EDS was found to be as high as 87.2% among OSA patients [2, 31]. In our patient cohort of a total of 93 individuals, EDS was found to be (ESS ≥ 14) as 46.5% ($n = 20$) among OSA patients ($n = 43$). In coherence with the literature data, it seems that EDS prevalence is nearly 50% or above among OSA patients.

In conclusion, we did not find an association between MAOA-uVNTR variants and excessive daytime sleepiness.

The necessity of further replications with multiple genes are beyond doubt since a single gene may play a minor role in the modulation and researches with some other genes together with MAOA in different sample populations may merit considerable attention.

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Compliance with ethical standards

Conflict of interest None of the authors has any conflict of interest to declare.

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