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Association study of a functional catechol-o-methyltransferase polymorphism and smoking in healthy Caucasian subjects

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Keywords: Catechol-o-methyltransferase polymorphism; Healthy subjects; Genetics; Smoking

Abstract

Tobacco smoking is a global health problem. The association of a functional common polymorphism in the catechol-o-methyltransferase gene (COMT val158met) with smoking behavior has been extensively studied, but with divergent findings. In the present study the frequency of COMT genotypes and alleles was evaluated in 578 male and a smaller group of 79 female unrelated, medication-free Caucasian healthy subjects of Croatian origin. Smokers were classified as subjects smoking ≤ 10 cigarettes per day, while subjects who never smoked in their life were regarded as nonsmokers. A χ^2 test with standardized residuals and Bonferroni correction revealed significant (P=0.0017) differences in *Met/Met, Met/Val* or *Val/Val* genotype frequency between male smokers and nonsmokers. This significant association between COMT val158met polymorphism and smoking was not detected in female subjects, due to small number of women, which represents a limitation of the study. Our results confirmed the significant association between COMT variants and smoking, which was due to the higher frequency of *Val/Val* homozygotes in male smokers compared to male nonsmokers. These results suggest that carriers of high activity COMT variant are more prone to develop a higher level of nicotine dependence, or that they release more dopamine than carriers of *Met/Met* or *Met/Val* genotypes.

Tobacco smoking is a global health problem with serious and far-reaching consequences [7]. Substances from tobacco smoke may affect brain functions via several neurotransmitter systems [7]. Smoking is related to different neuro-psychiatric disorders and altered behaviors, eliciting functional and structural changes in the brain, and might represent a risk factor for suicide [7]. Tobacco-related ailments, including heart diseases, pulmonary diseases, lung cancer and other malignancies, kill one in 10 adults [43]. According to the report on global smoking statistics issued by WHO in 2002, smoking will kill one in six people by the year 2030. One-third of adults in Croatia smoke, resulting in the smoking prevalence of 36%, which is higher than the respective prevalence of 33% in most European Union countries [22]. The First Croatian Health Project showed that 34% of males and 27% of females smoke regularly [40]. In addition, within the population of smokers in Croatia, 74% start smoking before the age of 20 [40].

Environmental, biological and genetic factors are involved in smoking behavior, smoking initiation, regular tobacco use, and nicotine dependence [15,19,27,38]. Dopamine is a neurotransmitter important in mediating drug reward. Many different drugs have the ability to alter dopamine concentration in reward-relevant regions of the brain [17]. Nicotine binds to nicotinic acetylcholine receptors in the brain and stimulates dopamine release and reuptake [30]. This leads to the increase in dopamine concentration [19]. Dopaminergic system, as well as allelic variations in genes involved in the dopamine pathways, is considered the major risk factor for tobacco use and development of nicotine dependence [17]. Candidate genes involved in nicotine dependence include genes for tyrosine hydroxylase (involved in dopamine synthesis), dopamine DRD1-DRD5 receptors; dopamine transporter (involved in dopamine reuptake), catechol-O-methyltransferase (COMT) and monoamine oxidase, and dopamine-beta-hydroxylase (enzymes for dopamine metabolism) [29,33].

COMT is an Mg^{2+}-dependent enzyme responsible for degrading catecholamine neurotransmitters (dopamine, epinephrine, norepinephrine) [41]. COMT converts dopamine into 3-methoxytyramine and norepinephrine into normetanephrine, by introducing a methyl group derived from S-adenosyl methionine to a hydroxyl group located on a catechol nucleus [1]. It has been associated with broad range of psychiatric disorders. *COMT* gene lies within the q11 band of human chromosome 22 [12], and has two promoters, namely P1 and P2 [36]. The longer mRNA transcribed by initiation through P2 promoter encodes for membrane-bound COMT (MB-COMT), and the transcription of shorter mRNA is directed by promoter P1 encoding for soluble COMT (S-COMT). The longer MB-COMT form is mainly expressed in brain neurons [24], and it has a higher affinity for the substrate, but a lower catalytic activity then the shorter form (S-COMT) [21]. The most studied variation in the *COMT* gene is a single base pair substitution of guanine for adenine which alters a single protein building block (amino acid) in the enzyme, replacing the amino acid valine (*Val*) with methionine (*Met*) at position 158 (Val158Met) in the longer form of the enzyme (MB-COMT), and at position 108 (Val108Met) in the shorter form (S-COMT) [21]. This Val108/158Met substitution or rs4680 is a functional polymorphism that affects COMT activity [16,21,42]. The *Val* variant of COMT is a high (H) activity allele, and has a higher stability and activity, catabolizing dopamine up to four times the rate of its *Met* alternative (or the low activity (L) allele) [4,34,41].

Several studies investigated the association between COMT Val158Met polymorphism and nicotine dependence with divergent findings. On one hand, no association between COMT polymorphism and smoking initiation, smoking persistence, or smoking cessation has been confirmed [6,11,25], while on the other hand, some studies show significant association between COMT Val158Met polymorphism and nicotine dependence

[2,13,32], or smoking cessation [5,28,29]. Sex differences can be observed in the genetic origins of smoking [2,10,20], and in the association between COMT Val158Met genotype and smoking behavior [2,5]. We tested the hypothesis that COMT Val158Met is associated with smoking in ethnically homogenous sample of healthy male and a smaller group of female Caucasian subjects of European ancestry, pertaining to Croatian origin.

The study included 578 male and 79 female unrelated, medication-free Caucasian healthy subjects of Croatian origin, who were recruited in the period between 2006 and 2009 at the University Hospital Centre Zagreb, Zagreb, Croatia, and who filled in the questionnaire answering the questions about their medical history, smoking and drinking habits. All subjects were categorized into smokers and nonsmokers, with smokers classified as subjects smoking ≤ 10 cigarettes per day, and nonsmokers classified as subjects who never smoked in their life. The average age of male and female subjects was 39.435±11.771 and 43.038±13.307 years, respectively. All individuals gave their detailed medical history. Inclusion criteria were no current medication therapy; no previous or current psychiatric disorders; no drug or alcohol abuse, nor suicidal attempts; no family history of psychiatric disorders (determined according to the answers of participants about the mental health status of their parents, grandparents, siblings and children); not being related; and belonging to the native ethnic group with at least three generations living in the region. Written informed consent was obtained from all participants, after explaining the aims and procedures of the study, under guidelines approved by the Ethics committee of the University Hospital Centre Zagreb, Zagreb, Croatia. All human studies have been executed with the full cooperation of participants, adequate understanding, and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Blood samples (8 ml) were drawn using plastic syringes with 2 ml of acid citrate dextrose anticoagulant at 08.00 h. Genomic DNA was extracted from peripheral blood using the salting

out method [26]. The COMT val158met polymorphisms was genotyped with the ABI Prism 7000 Sequencing Detection System apparatus (ABI) using the Taqman-based allele-specific polymerase chain reaction assay, according to manufacturers instructions (Applied Biosystems, Foster City, CA, USA). The primers and probes were purchased from Applied Biosystems. The results were expressed as means ± standard deviations (S.D.) and evaluated with Sigma Stat 3.5 (Jandell Scientific Corp. San Raphael, California, USA) and Microsoft Excel. Differences in age were assessed using one-way analysis of variance (ANOVA). The Hardy-Weinberg analysis was used to test the equilibrium of the population. The differences in genotype and allele frequencies were evaluated using the χ^2 test, with standardized residuals (R) in each cell. Bonferroni correction, with the number of tests (N=2) as correction factor, was used, and the level of significance was set to $\alpha = 0.025$.

The genotype (*Met/Met, Met/Val* or *Val/Val*) distribution in male (χ^2=0.068; *d.f.*=1; *P*=0.795) or female (χ^2=0.293; *d.f.*=1; *P*=0.588) subjects, as well as in male smokers (χ^2 =3.450; *d.f.*=1; *P*=0.063) or male nonsmokers (χ^2 =2.576; *d.f.*=1; *P*=0.108) did not deviate significantly from the expected Hardy–Weinberg equilibrium. The same distribution was observed in female smokers (χ^2 =0.631; *d.f.*=1; *P*=0.426) or female nonsmokers (χ^2 =1.142; *d.f.*=1; *P*=0.285). In line with previous findings [12,24], this equilibrium showed a marginal trend towards significance in male smokers.

Due to the fact that gender-gene interaction significantly affects smoking behavior, and smoking was reported to be differently related with COMT val158met polymorphism in female and male subjects [2,5], the effect of smoking was evaluated separately in male and female subjects (Table 1).

Within larger group of male smokers and nonsmokers (Table 1), significant difference was found in *Met/Met, Met/Val* and *Val/Val* genotype frequency, but not in *Met* or *Val* allele frequency. These differences were due to the higher frequency of *Val/Val* genotypes (32.8%

vs. 22.6%) in male smokers compared to male nonsmokers. The highest R value of 1.85 was detected in *Val/Val* genotype in smokers, indicating significant association of the *Val/Val* genotype with smoking behavior in male subjects. In the smaller group of female smokers and nonsmokers (Table 1), there was no significant difference in genotype and allele frequency of the COMT val158met.

Female nonsmokers were slightly older than male smokers, but this difference was not significant after the Bonferroni correction. Age of the other groups did not differ significantly (Table 1). There was no significant difference in age between groups of male (F=1.080; $d.f.$=5,572; P=0.371) and female (F=0.640; $d.f.$=5,73; P=0.670) healthy subjects subdivided according to smoking status and COMT genotype.

The results of the present study indicate a significant association between smoking (smoking more than 10 cigarettes per day, versus never smoking) and COMT val158met polymorphism in large groups of male homogenous Caucasian individuals of Croatian origin. This strong association detected for *Met/Met, Met/Val* and *Val/Val* genotype frequency, significant even after a conservative Bonferroni correction, was due to the higher percent of *Val/Val* homozygotes in male smokers compared to male nonsmokers. The difference in genotype distribution was modest (a 10% difference between male nonsmokers and smokers), showing that *Val/Val* genotype occurs more frequently among male smokers than nonsmokers, and indicating its contribution to this significant association.

Our results concur with the data showing an association between the *Val/Val* genotype and smoking at least one pack per day [37], *Val* allele and persistent smoking among women [5], and *Val* allele and current smoking status [10]. Additionaly, *Val* carriers were reported to relapse quicker into smoking in randomized treatment trials of smoking cessation [28]. On the other hand, collective results of our study are in disagreement with previous findings exhibiting association of the low activity *Met* allele with nicotine dependence [2], or with the

results indicating that one or more *Met* alleles may enhance the addictive power of nicotine [32]. Additionally, our data does not coincide with the finding that *Met* allele carriers had higher Fagerstrom Test for Nicotine Dependence and started to smoke earlier, indicating that *COMT* polymorphism influences smoking severity and age of onset, which was initially observed in Chinese male population [13]. Finally, our findings are not in line with the results showing no association between *COMT* polymorphism and smoking initiation, smoking persistence, smoking cessation or smoking intensity [6,11,13,25,35].

The positive association between *Val/Val* genotype and smoking, found in the present study, was not confirmed in female subjects due to the substantially smaller female sample size, presumably due to the type I error, which represents a limitation of the study. In contrast to our results, previous studies reported association of *Met/Met* genotype and higher probability of smoking cessation in women [5]. The genetic origin of smoking, smoking initiation and smoking persistence is gender dependent [2,10,20], indicating that the COMT val158met polymorphism affects catecholaminergic systems differently in male and female patients. A possible explanation for this gender-specific association might be due to the effect of estrogen in females, which modulates neurotransmission and neuronal excitability of catecholaminergic systems [14], resulting in different levels of dopamine in the synapse and different influence of dopamine on emotion and motivation among men and women [7]. In addition, it has been suggested that gender related differences in smoking behavior might be due to the fact that COMT val158met polymorphism may differently influence personality traits that presumably affect smoking behavior, and that *Val/Val* genotype may be related to higher novelty or risk seeking traits than carriers of other genotypes [7,10,39]. Since no evaluation of personality traits in male subjects was performed in the present study, we can neither confirm nor exclude these presumptions. The data, showing increased tendency towards smoking in *Val/Val* male homozygotes, might be explained by the fact that these

subjects, who have high activity COMT variant, are more prone to develop a higher level of dependence and are more predisposed to smoking relapse [5,31]. This effect might be mediated by the lower concentration of frontocortical dopamine in carriers of the high activity COMT variant [9]. Significant association between *Val/Val* genotype and smoking might also be due to the fact that the *Val/Val* genotype may represent a risk factor for altered brain function and cognition during nicotine abstinence, i.e. a risk factor for increased susceptibility to nicotine dependence and smoking relapse [23]. In addition, carriers of *Val/Val* genotype were shown to have greater release of dopamine induced by smoking than carriers of *Met/Met* or *Met/Val* genotypes [3].

In conclusion, the results of the study confirmed a significant association between COMT variants and smoking more than 10 cigarettes per day, compared to complete, life-long abstinence from smoking in healthy male medication-free Caucasian healthy subjects. The observed association was due to the higher percent of *Val/Val* homozygotes in male smokers compared to male nonsmokers.

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Table 1. Age and the COMT genotype and allele number (N), frequencies (%), and standardized residuals (R) in male and female healthy subjects, subdivided according to the smoking status.

	Healthy subjects (N=657)							
	Men				Women			
	Smokers		Nonsmokers		Smokers		Nonsmokers	
	N (%)	R	N (%)	R	N (%)	R	N (%)	R
Genotypes								
Met/Met	44 (24.4)	0.20	93 (23.4)	-0.14	3 (13.0)	-1.08	16 (28.6)	0.69
Met/Val	77 (42.8)	-1.46	215 (54.0)	0.98	13 (56.5)	0.68	24 (42.8)	-0.44
Val/Val	59 (32.8)	**1.85**	90 (22.6)	-1.24	7 (30.5)	0.12	16 (28.6)	-0.08
χ^2*-test*	χ^2 =8.130; *d.f.*=2; ***P*=0.017**				χ^2 =2.318; *d.f.*=2; *P=0.316*; NS			
Alleles								
Met	165 (45.8)	-0.89	401 (50.4)	0.55	19 (41.3)	-0.71	56 (50.0)	0.32
Val	195 (54.2)	0.79	395 (49.6)	-0.58	27 (58.7)	0.58	56 (50.0)	-0. 43
χ^2*-test*	χ^2=1.870; *d.f.*=1; *P=0.171*; NS				χ^2=0.671; *d.f.*=1; *P*=0.413; NS			

	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Age (years)	37.82	11.00	42.30	12.54	40.15	12.04	43.34	13.71**
ANOVA	*F*=3.387; *d.f.*=3,653; *P*=0.018; ***P*=0.017 vs. male smokers							

N is the observed number of genotypes or alleles; % is the frequency of the genotypes or alleles; R represents the standardized residuals in each cell; NS = not significant