Lack of association between the Val158Met catechol-*O*-methyltransferase gene polymorphism and methamphetamine dependence

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Abstract

OBJECTIVES: About 25 000 serious methamphetamine abusers live in the Czech Republic among the total population of 10 million. Dependence on methamphetamine is markedly related to the brain neurotransmitter dopamine, metabolised by catechol-*O*-methyltransferase enzyme. The main aim of the study was to ascertain whether the Val158Met catechol-*O*-methyltransferase gene polymorphism is associated with methamphetamine dependence in this Central European country.
METHODS: One hundred and twenty-three subjects dependent on methamphetamine (women N=44), parents of sixty-seven dependent individuals, and four hundred healthy controls (women N=250) were involved into the study. We performed a population-based as well as family-based genetic association studies.
RESULTS: We did not find any significant association between the Val158Met catechol-*O*-methyltransferase gene polymorphism and methamphetamine dependence using the population-based or family-based design (p=0.41–0.66; Chi-Square Test or UNPHASED program, Version 3.1.4, respectively). We found a trend toward a statistically significant difference between the Val allele carriers and Met/Met homozygotes in the frequence of psychotic symptoms induced by methamphetamine (more frequent in Val carriers; p=0.062; Chi-Square Test).
CONCLUSION: Further research involving haplotype analysis and other dopamine-related genetic polymorphisms in large populations is needed. More attention should also be paid to possible role of the Val158Met catechol-*O*-methyltransferase gene polymorphism in individual clinical subtypes of dependence on methamphetamine involving e.g. psychotic features or violence.

ORIGINAL ARTICLE

INTRODUCTION

Methamphetamine (MA) was first synthesized from ephedrine in Japan in 1893 by chemist Nagai Nagayoshi (http://en.wikipedia.org 2011). Methamphetamine is a central nervous system stimulant having a high potential for abuse. It increases the release and blocks the reuptake of dopamine, leading to high levels of dopamine in the brain. Dopamine is involved in reward, motivation, the experience of pleasure, and motor function. Methamphetamine's ability to release dopamine rapidly in reward regions of the brain such as ventral striatum produces intense euphoria (Drevets *et al.* 2001). Chronic methamphetamine abuse significantly changes both the brain structure and its functions. Neuronal apoptosis and nerve terminal degeneration are the main consequences of MA neurotoxicity (Schep *et al.* 2010).

United Nations Office on Drugs and Crime estimates that between 13.7 and 52.9 million people worldwide used amphetamine-group substances at least once in 2009, with a corresponding annual prevalence range of 0.3% to 1.2% of the population aged 15 to 64 years (United Nations Office on Drugs and Crime 2010).

In the United States approximately 10 million people 12 years and older have abused methamphetamine in their lifetimes (http://www.nida.nih.gov 2010). The number of serious illegal drug abusers amounts to 37 000 in the Czech Republic, where the total number of population equals to 10 million. Methamphetamine abusers represent about 25 000 subjects (Government office of the Czech Republic 2010).

Catechol-*O*-methyltransferase (COMT) catalyses methyl conjugation and thus inactivation of catecholamine neurotransmitters such as dopamine and norepinephrine (Lachman *et al.* 1996). In the brain, the role of COMT is restricted mainly to the prefrontal cortex. COMT is encoded by a gene localized on chromosome 22q11.1-11.2. Two codominant alleles (G and A) in exon 4 of the COMT gene influence the amino acid structure (Val or Met) at codon 158. The COMT enzyme activity is genetically polymorphic (high activity in Val/Val genotype, intermediate activity in Val/Met genotype, and low activity in Met/Met genotype). The difference in COMT activity is three to four-fold (Val/Val vs Met/Met). This single nucleotide polymorphism (SNP) is commonly referred to as Val158Met, or rs4680 polymorphism (Malhotra *et al.* 2002). Thus the Val158Met polymorphism of the COMT gene is functional, easily detectable, and significantly related to metabolism of catecholamines, which underlie pathogenesis of many mental disorders.

Because dopamine activity in the human brain is influenced by methamphetamine, research into the Val-158Met COMT genetic polymorphism in MA dependence is warranted.

The aim of the study was to ascertain whether the Val158Met COMT gene polymorphism is associated with methamphetamine dependence in the Czech Republic. We performed a genetic association study in a case-control as well as family based design (family-based association test, FBAT). Furthermore, we investigated whether the Val158Met COMT gene polymorphism is associated with psychosis induced by methamphetamine.

SUBJECTS AND METHODS

Subjects dependent on methamphetamine

Patients dependent on methamphetamine (DSM-IV Code 304.40) hospitalised and treated at the Addiction Treatment Unit in Nechanice in 2007–2010 agreed to participate in the investigation. Addiction to substances other than MA, apart from nicotine, was an exclusion criterion. Possible psychotic disorders induced by methamphetamine (DSM-IV Codes 292.11 and 292.12) in each patient´s medical history were also recorded. All diagnostic assessments were made by the first author of the study, who is an experienced psychiatrist. In addition to the patients, at least one of the parents in every subject was also offered to be genotyped for the Val158Met COMT gene polymorphism for the purpose of FBAT.

Healthy controls

Healthy volunteers were recruited through the Laboratory of Neurobiology and Molecular Psychiatry in Brno. The control subjects were nonsmokers, and did not use any illicit drug. Alcoholism was excluded using the Michigan Alcohol Screening Test (MAST; Selzer 1971) and the "Cut down, Annoyed, Guilty, Early morning drink" (CAGE; Ewing 1984) questionnaires. We excluded psychiatric illness through an interview with an experienced psychiatrist.

Val158Met genotyping

DNA was extracted from peripheral blood leukocytes using QIAamp Blood Mini Kit (Qiagen, Germany) (Beranek *et al.* 2004). Polymerase chain reaction (PCR) was performed in glass capillaries in a total reaction volume of 20 µl (LightCycler 1.5, Roche Diagnostics, Germany). The reaction mixture contained 5 pmol of each primer (5´-CTCATCACCATCGAGATCAA-3´ and 5´-GATGACCCTGGTGATAGTGG-3´), 1 pmol of hybridization probes (sensor, 5´-TCACGCCAGC-GAAATCCAC-Fluo-3´; anchor, 5´-LCRed640-TCC-GCTGGGTGATGGCG-Pho-3´), 2 mM $MgCl_2$, 1 µl of LightCycler FastStart DNA Master Hybridization Probes (Roche Diagnostics, Germany), and 50 ng of genomic DNA. The cycling profile was as follows: one cycle consisting of 95 °C for 10 minutes, 45 cycles consisting of 95 °C for 2 seconds, 55 °C for 5 seconds, and 72 °C for 5 seconds. After amplification, the melting analysis was carried out by denaturation at 95 °C for 30 seconds, annealing at 40 °C for 90 seconds, and a slow increasing the temperature to 75 °C with a ramp rate of 0.1 °C/second. The Met and Val alleles produced melting temperature peaks at 59 °C and 64 °C, respectively.

Statistical analysis

We used the NCSS 2007 statistical software to test the differences in the Val158Met COMT allele and genotype distribution between the patients and healthy volunteers, as well as the influence of the Val158Met COMT genotype on possible psychotic features induced by methamphetamine (Chi-Square Test, Fisher´s Exact Test). Likelihood-based association analysis for nuclear families with missing genotype data implemented in the program UNPHASED, Version 3.1.4 (Dudbridge 2008) was performed for the purpose of family-based association test. Here the control group is created from non-transmitted parental alleles. This approach allows for close matching of the case and controls groups with respect to ethnic stratification as well as other confounders such as socioeconomic status. Parental phenotype information is not required and is not used (Falk & Rubinstein 1987).

Ethical issues

The study was approved by the Ethics Committees of the Faculty of Medicine, Charles University in Hradec Kralove, and the 1st Faculty of Medicine, Charles University in Prague. The protocol for the research project conforms to the provisions of the Declaration of Helsinki in 1975 (as revised in 1983). The patients, their parents as well as healthy controls voluntarily signed the "informed consent". The anonymity of all participants is preserved.

RESULTS

Patients

One hundred and twenty-three subjects (women N=44) at an average age of 24.0±4.4 years (range 18–38 years) dependent on methamphetamine represented the group of patients. The average duration of methamphetamine abuse was 7.0±3.1 years (range 1–15 years). Other demographic and clinical data are presented in Table 1. Sixty-seven patients were eligible for the family-based association test, when the COMT genotyping was perfomed in both parents (N=32) or at least one of them (N=35).

Healthy controls

Four hundred individuals (women N=250) not dependent on any illicit substance, nicotine or alcohol, suffering from no serious physical or mental disorder, at an average age of 32.0±11.1 years (range 18–66 years) were used as healthy controls. They were recruited at medical units of general hospitals, transfusion units, Czech army, or at local universities in Brno and Prague.

Population-based genetic association study

The genotype frequencies of the Val158Met COMT gene polymorphism in subjects dependent on methamphetamine as well as healthy controls did not deviate from the Hardy-Weinberg equilibrium (patients $\chi^2 = 1.81$, $p>0.05$; controls $\chi^2 = 0.1$, $p>0.05$). Neither genotype ($p=0.41$) nor allele ($p=0.51$) frequencies related to the Val158Met COMT gene polymorphism differed significantly between the patients dependent on methamphetamine and healthy volunteers (Odds Ratio (OR) = 1.006; 95%CI = 0.76–1.34; Risk Ratio (RR) = 1.003; 95%CI = 0.87–1.15) (Table 2).

Tab. 1. Selected demographic and clinical data on the patients dependent on methamphetamine (N=123).

Variable	Option	N	%
Education	Junior high school	47	38%
	Apprenticeship	53	43%
	High school	23	19%
Employment	Paid job	37	30%
	Unemployed	86	70%
Marital status	Single	107	87%
	Divorced	13	11%
	Married	3	2%
Housing	With parents or grandparents	98	80%
	Independent housing	20	16%
	Homeless	5	4%
Criminal behavior in the history	Yes	52	42%
	No	71	58%
Mode of MA application	I.V.	104	85%
	Sniffing	19	15%
Common MA dose in one application	0.5 gram	61	50%
	1 gram	29	24%
	Other doses	33	26%
Frequency of MA application	Once a day	63	51%
	Several times a day	18	15%
	Less frequently than once a day	42	34%
History of psychotic symptoms	Yes	35	28%
	No	88	72%

Tab. 2. Val158Met COMT genotypes in methamphetamine dependence and healthy controls.

Subjects	Met/Met (N)	Val/Met (N)	Val/Val (N)	Total (N)
Methamphetamine dependence	36	54	33	123
Controls	104	203	93	400

Genotype frequencies in the patients vs controls: $p=0.41$ (Chi-Square Test)
Allele frequencies in the patients vs controls: $p=0.51$ (Fisher´s Exact Test)

Family-based genetic association study

We did not find a significant difference between observed and expected transmission frequencies of either Val (transmissions N=64, expected =68) or Met alleles (transmissions N=70, expected =66) from the parents to the patients dependent on methamphetamine (p=0.66, Met allele OR = 1.13; 95%CI = 0.68–1.87).

Association between the Val158Met COMT gene polymorphism and psychotic features in methamphetamine dependence

Neither genotype nor allele frequencies of the Val-158Met COMT gene polymorphism were significantly different in psychotic (N=35) vs non-psychotic (N=88) patients (p=N.S., Chi-Square Test). We only found a trend toward a statistically significant difference between the Val allele carriers and the Met/Met homozygotes (p=0.062, Chi-Square Test), when the Val allele was more frequent in psychotic vs non-psychotic patients (Table 3).

DISCUSSION

Neither genotype nor allele frequencies related to the Val158Met COMT gene polymorphism differed significantly between the patients dependent on methamphetamine and healthy volunteers in our study. Our results may have been influenced by a gender effect, when women represent about one third of the patients but prevail in the control group. In women, COMT is required for the detoxification of catecholestrogens, and estrogen has been observed to inhibit COMT gene transcription. That is why future studies should also be stratified by gender (Hosak 2007). We also did not find a significant difference in the transmission frequency of the Val allele versus the Met allele from the parents to the patients dependent on methamphetamine. Neither genotype nor allele frequencies of the Val158Met COMT gene polymorphism were significantly different in psychotic vs non-psychotic patients. Our finding of a trend toward a statistical significance in the relation of the Val allele to psychosis in the patients only contributes to conflicting results in relation to the role of the Val158Met COMT gene polymorphism in psychotic symptoms. A presumable explanation is that this polymorphism does not directly influence psychosis in its entirety, but rather impacts on individual psychotic symptoms as violent behavior or cognitive weakening (Hosak 2007).

Table 3. Val158Met COMT genotypes in methamphetamine dependence with vs without psychotic features induced by methamphetamine.

Genotype	Psychotic patients (N)	Non-psychotic patients (N)	Total (N)
Val/Val + Val/Met	29	58	87
Met/Met	6	30	36
Total	35	88	123

Val allele frequency in the patients with vs without psychosis: p=0.062 (Chi-Square Test)

One of the key works that stimulated the interest of researchers into the role of the Val158Met COMT gene polymorphism in substance abuse was published by Tiihonen *et al.* (1999). The results indicated that the COMT polymorphism, explicitly its Met allele, contributes sigificantly (p=0.009) to the development of late-onset alcoholism. Even if the Tiihonen´s work has been published as far back as 12 years ago, only a few subsequent studies were directly aimed at the COMT polymorphism in methamphetamine dependence. Having used the PubMed search with the key words "methamphetamine AND polymorphism AND COMT" on the 22nd of February, 2011, we obtained nine references, with only three fully relevant to the topic (Li *et al.* 2004; Bousman *et al.* 2009; Bousman *et al.* 2010).

Li *et al.* (2004) analyzed the Val158Met COMT gene polymorphism and the 120-bp variable number of tandem repeats (VNTR) polymorphism in the promoter of the dopamine D4 receptor gene for association with methamphetamine abuse in 416 MA abusing subjects and 435 normal controls of the Han Chinese origin in Taiwan. They found an excess of the high activity Val allele in the MA abuser group, consistent with several previous reports of association of this allele with drug abuse. They also discovered significant (p=0.0003–0.01) interactions between the COMT and dopamine D4 receptor gene polymorphisms. The authors concluded that genetic variation in the dopamine system may encode an additive effect on risk of becoming a methamphetamine abuser.

Bousman *et al.* (2010) genotyped 117 methamphetamine dependent males and 76 controls of Caucasian origin for variants located in six genes (AKT1, ARRB2, BDNF, COMT, GSTP1 and OPRM1) previously found to be associated with MA dependence in Asian populations. None of the putative gene associations was significantly replicated in this sample of Caucasian men. The authors emphasize the role of ethnic divergence in genetic associations in methamphetamine dependence.

According to Bousman *et al.* (2009) who have reviewed 38 genetic association studies of MA use disorders, the COMT gene is associated with methamphetamine abuse, but not dependence or MA psychosis. Similar to other behavioral, psychiatric, and substance use disorders, the genetic epidemiology of MA use disorders is complex and likely multifactorial. National and international collaborative efforts are needed to increase the availability of large population-based samples and improve the power to detect genetic associations of small magnitude.

Lohoff *et al.* (2008) genotyped 330 cocaine dependent individuals and 255 normal controls of African descent in the U.S. for three single nucleotide polymorphisms (SNPs) in the COMT gene. Haplotype analysis showed a significant association for a two-marker haplotype rs737865-Val158Met with the dependence on cocaine (p=0.005). According to the authors, the 158Met allele, both in itself or as a part of a haplotype, might have functional effects on dopamine-derived reward processes and cortical functions resulting in increased susceptibility for cocaine dependence.

Based on a study of DNA samples from 45 populations for 63 SNPs across the region of 22q11.2 encompassing the COMT gene Mukherjee *et al.* (2010) suggest that not the Val158Met polymorphism itself but its haplotypic combinations with other alleles in the promoter region and in the 3´-untranslated region are responsible for the associations with various mental disorders including substance dependence. Future association studies should be based on the SNPs that define the common haplotypes in the studied population.

Kweon *et al.* (2005) did not find any association of the Val158Met COMT gene polymorphism with alcoholism in 97 male alcoholics and 94 male normal controls in Korea. However, the differences between the violent and non-violent groups were significant in terms of the frequencies of the COMT genotypes (p=0.019) and the alleles (p=0.012). This suggests that the Val158Met COMT polymorphism is not associated with the development of alcohol dependence, but may affect the susceptibility to a clinical heterogeneity of this mental disorder. This may also be true for methamphetamine dependence. Samochowiec *et al.* (2006) in a family-based and case-control study in 100 Polish families with alcohol dependence and 196 healthy control subjects similarly did not find any influence of the Val158Met COMT gene polymorphism on alcoholism as a whole, but support the hypothesis that there are various subtypes of alcohol dependence, which differ depending on their genetic background. As for dependence on methamphetamine, suitable clinical subtypes may be related e.g. to psychotic symptoms or violence.

CONCLUSION

We did not find any significant association between the Val158Met catechol-*O*-methyltransferase gene polymorphism and methamphetamine dependence using the population-based or family-based design. The role of the Val158Met COMT gene polymorphism in methamphetamine dependence is probably not fundamental, but may be related to clinical subtypes of this mental disorder.

In spite of substantial interest in genetics of substance dependence, studies of methamphetamine dependence are only few including those investigating the Val-158Met COMT gene polymorphism. Further research involving haplotype analysis and other genetic variations in dopamine system in large population-based samples with a respect to ethnic genetic divergence is needed. More attention should also be paid to possible clinical subtypes of dependence on methamphetamine.

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