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Effect of rs4680 (Val^{158}Met) Polymorphism of the *COMT* Gene on Opioid Addiction in an Iranian Population: A Case-Control Study

Fatemeh Dahmardeh [1,*] and Alireza Rezaeifar [2]

[1]Department of Biology, Faculty of Sciences, University of Zabol, Zabol, Iran
[2]Department of Clinical Biochemistry, School of Medicine, Zabol University of Medical Sciences, Zabol, Iran

[*]*Corresponding author*: Department of Biology, Faculty of Sciences, University of Zabol, Zabol, Iran. Email: ft_dahmardeh@yahoo.com

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Abstract

Background: Drug addiction is a serious neurological disorder that is significantly associated with mortality and morbidity. It is accompanied by social, economic, and health problems for both the individual and the whole society. Genes playing a role in the catabolism of dopamine (DA), such as catechol-O-methyltransferase (*COMT*), seem to be plausible candidate genes for drug dependence.
Objectives: This study aimed to investigate the effect of rs4680 (Val^{158}Met) polymorphism of the *COMT* gene on opioid addiction (OA) in the whole samples and male/female subsamples of an Iranian population.
Methods: The current case-control study was conducted with 96 cases (87 men and nine women with OA) and 142 controls (117 men and 25 women). Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) was used to genotype the samples.
Results: Our results showed no significant association between the *COMT* rs4680 gene polymorphism and OA in the total population. In addition, we found no notable correlation between rs4880 and OA in male and female subsamples.
Conclusions: In conclusion, there was no correlation between the rs4680 polymorphism of the *COMT* gene and opioid addiction. The conflicting results in studies with different ethnicities and sample sizes suggest that additional studies are needed to investigate the Val^{158}Met polymorphism correlation with other variants of the *COMT* gene in larger populations and in both genders with opioid and other addictions.

Keywords: Addiction, Dopamine, *COMT* Gene, rs4680, Polymorphism, Drug Abuse

1. Background

Substance abuse is a universal health and social problem. According to the available information, the global prevalence of opioid users is estimated to be approximately 32.4 million, accounting for 0.7% of the world's adult population (aged 15 - 64 years) (1). Opioids are the most popular drugs of use in Iran because of a long border with the main producer of illicit opioids, Afghanistan (2). Opioids refer to drugs extracted from the opium poppy, including morphine, codeine, and derivatives like heroin (1). Opioid addiction (OA) is a chronic disease that can affect the brain and behavior (3). OA is significantly associated with mortality and morbidity and is accompanied by social, economic, and health problems for both the individual and the whole society (4).

Several family, adoption, and twin studies have identified the notable role of heritable determinants on individual differences in addiction (5). Previous studies have shown that genetic factors may constitute 30% - 60% of risk factors for drug addiction (6). It has been suggested that genes involved in molecular events and altered due to drug abuse are the first candidates for identifying specific genes involved in drug addiction (7). For example, prolonged drug abuse alters the levels of dopamine (DA), a kind of neurotransmitter in the dopaminergic system, which plays an important role in drug reward and has been assumed to underlie addiction development and permanence (8).

The critical mechanism to control the levels of synaptic dopamine is the degradation of DA by the enzyme Catechol-O-methyltransferase (COMT). The COMT is an important enzyme to metabolize catechol neurotransmitters such as DA and regulates the level of DA, especially in the frontal cortical areas of the brain. The COMT enzyme deactivates DA by methylation (9). In humans, there are two isoforms of COMT, soluble form (S-COMT) and membrane-bound form (MB-COMT), which are formed through alter-

native splicing. The MB-COMT is 50 amino acids longer than the S-COMT and prevails in the brain (10). It has a higher catechol affinity than the S-form; thus, MB-COMT is able to terminate the dopaminergic neurotransmission at low concentrations of catecholamines (9).

Linkage studies have identified many genetic determinants of addictive diseases and genetic variants contributing to opiate addiction (7). Furthermore, the relationship of *COMT* gene polymorphisms and psychiatric disorders (11) with alcohol (12) and drug dependence (13) in different populations was reported previously. However, there are a few studies on the association between rs4680 (Val^{158}Met) polymorphism of the *COMT* gene and drug dependence (14).

2. Objectives

Considering the crucial role of *COMT* in DA metabolism, as a major contributor to the etiology of drug dependence, the present study was designed to evaluate the effect of *COMT* rs4880 polymorphism (common missense SNP) on opiate addiction in both the whole sample and the male/female subsamples in the Southeast population of Iran (Zabol city).

3. Methods

3.1. Subjects

In this study, 96 people with OA (87 men, nine women) were recruited from a drug addiction treatment center in Zabol city, Sistan and Baluchistan province, Iran. They all met the criteria for drug dependence based on the Diagnostic and Statistical Manual of Mental Disorders and the International Statistical Classification of Diseases. The subjects suffering a major psychiatric disorder with possible involvement of the neurotransmitter systems under study were excluded (12, 15). In addition, 117 men and 25 women were selected as the control group that self-reported no history of drug abuse and major psychiatric disorders.

This study was approved by the Ethics Committee of the University of Zabol (IR.UOZ.REC.1393.01) and performed in accordance with the Declaration of Helsinki.

3.2. DNA Extraction and Genotyping

Blood samples of volunteers participated in the study were collected and stored at -20°C until further experiments. The genomic DNA extraction was conducted by the salting-out method.

The polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied to genotype the samples. The favorable segment of the *COMT* gene with 628 bp in length was amplified by 5'-CGACTTAGTACATCCTTC-3' and 5'-GAGTAGAATCTTGGCTAG-3' forward and reverse primers, respectively. The primers were designed by the beacon designer program. Polymerase chain reaction (PCR) was done by routine PCR Mastermix (Genetbio, Denmark) based on the manufacturer's recommended procedure. PCR thermocycling conditions were as follows: denaturation at 94°C for 1 min, annealing at 51.8°C for 30 s, and extension at 72°C for 50 s for 34 cycles. The PCR products were digested with the restriction enzyme (*MSPI*-Roche diagnostics, Swiss). The digested DNA products were electrophoresed on a 2% agarose gel and stained with the green viewer. Gel images were taken by a Life Technologies E-gel imager instrument. The resulting fragments are shown in Figure 1. We repeated the genotyping for random samples (~20% of the total samples) and did not observe any genotyping error.

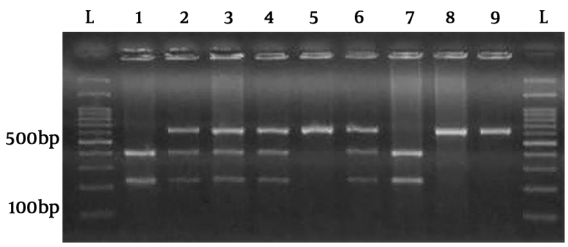

Figure 1. The PCR-RFLP results for COMT rs4680 polymorphism; L: 100-bp DNA ladder; 1 and 7: GG (225 and 403 bp); 2, 3, 4, 6: GA (225, 443, and 625 bp); 5, 8, 9: AA (625 bp)

3.3. Statistical Analysis

All statistical analyses were done by SPSS version 20.0. The logistic regression analysis was used to examine the association between rs4680 polymorphism and OA by calculating a 95% confidence interval (CI) and odds ratio (OR). The values of $P < 0.05$ were considered statistically significant.

4. Results

Table 1 presents the frequency of the rs4680 *COMT* genotype in the total population, females, and males. The observed genotypes in the total population were found in Hardy Weinberg equilibrium.

As shown in Table 2, the frequency of GG (ancestral), GA, and AA genotypes of *COMT* rs4680 was 20.8%, 57.3%, and 21.9% in cases and 25.4%, 40.8%, and 33.8% in controls, respectively. No significant association was found between *COMT* rs4680 gene polymorphism and OA in the total population. The frequency of AA genotype versus GG + GA in

Table 1. *COMT* rs4680 Genotype Frequency[a]

Genotype	Opioids Dependence			Controls		
	Male, N = 87	Female, N = 9	Total, N = 96	Male, N = 117	Female, N = 25	Total, N = 142
GG	19 (21.8)	1 (11.1)	20 (20.8)	31 (26.5)	5 (20.0)	36 (25.4)
GA	47 (54.0)	8 (88.9)	55 (57.3)	50 (42.7)	8 (32.0)	58 (40.8)
AA	21 (24.1)	0	21 (21.9)	36 (30.8)	12 (48.0)	48 (33.8)

[a]Values are expressed as No. (%).

the recessive model was higher in controls than in the OA group, but the difference was marginally insignificant (P = 0.06).

In addition, the logistic regression analysis showed no notable correlation between rs4880 and OA in female and male subsamples (Tables 3 and 4).

5. Discussion

Drug addiction is a serious neurological disorder influenced by many factors including genetic, behavioral, and environmental factors (16). Opioids are a major series of lethal substances. According to the National Institute on Drug Abuse reports, the death rate involving opioid drugs and drug overdose has increased about six to eight folds in recent decades (17).

Due to the critical role of DA pathways in drug reward, the genes involved in DA neurotransmission are the probable candidates for correlation with drug addiction (5). However, there are discrepant results in dopamine receptor and transporter genes in genetic association studies due to small sample size and complexity of the phenotype (18). Although many gene variants have been found to be risk factors for drug dependence (16, 19, 20), a few studies have examined associations between variation in the *COMT* gene and opioid addiction.

The COMT enzyme is a major regulator of the synaptic DA levels and plays an important role in DA catabolism. The human *COMT* gene is located on chromosome 22q11. The rs4680 polymorphism in the fourth exon of the *COMT* gene leads the substitution of Met for Val in residue 158 ($Val^{158}Met$) of its protein (21). It has been suggested that rs4680 may alter enzyme activity and subsequently, the efficiency of DA degradation and vulnerability to substance dependence. It has been shown that the COMT enzyme with Val/Val genotype leads to a three to four-fold increase in enzymatic activity as compared to Met/Met genotype (22, 23). Therefore, the rs4680 polymorphism of the *COMT* gene is a plausible marker for the genetic predisposition to addiction. To our knowledge, this is the first study investigating the effect of *COMT* $Val^{158}Met$ polymorphism on opioid addiction in an Iranian population.

The results of a recent meta-analysis by Taylor revealed a significant association between *COMT* $Val^{158}Met$ polymorphism and different psychiatric disorders (24). Similar results showed the contribution of rs4680 to susceptibility to schizophrenia and bipolar mood disorder in southwest Iran (25), but no association was found between rs4680 and schizophrenia in southeast Iran (26).

Controversial results have also been reported when evaluating rs4680 SNP in relation to addictive diseases. Horowitz et al. found an association between the G (Val) allele and heroin addiction (27). Lohoff et al. found a statistically significant difference between cocaine-dependent individuals (f (Met) = 35%) and normal controls (f (Met) = 27%) (P = 0.004) of African descent (28). Similar results on the implication of $Val^{158}Met$ polymorphism in a variety of substance abuse disorders were reported by several previous studies (29-31). However, no similar finding was shown by others (32-34). Moreover, Cao et al. observed no differences in genotype or allele frequencies for the rs4680 polymorphism between opiate-dependent cases and controls (35). The results from Voisey et al.'s study showed a weak association between rs4680 SNP and alcohol dependence (at the allele level), but it was not associated with nicotine or opiate dependence (12). Omidvar et al. demonstrated that GG (Val/Val) genotype of the *COMT* gene was associated with smoking cessation. However, they found no sex difference and no effect of the *COMT* polymorphism on smoking initiation (13). Christoffersen et al. showed that the AA genotype of rs4680 is less frequent in deceased patients with OA than in living patients with OA in Caucasian subjects (14).

Since different results have been observed when evaluating the effect of $Val^{158}Met$ SNP on behavioral disorders in males and females, it has been suggested that the $Val^{158}Met$ substitution has gender-specific implications (36). Therefore, in the current study, we conducted the association tests in both genders; however, our results showed no significant association between rs4680 SNP and OA in males and females. Similar to our results, Demetrovics et al. observed that the genotype frequency of *COMT* $Val^{158}Met$ did not differ between Hungarian opiate-dependent patients and controls (in the whole population and male/female

Table 2. The Frequency of Alleles and Genotypes of *COMT* rs4680 Polymorphism in the Case and Control Groups (Total Study Population)[a]

COMT, rs4680	Case, N = 96	Control, N = 142	P Value	OR (95% CI)
GG	20 (20.8)	36 (25.4)		1
GA	55 (57.3)	58 (40.8)	0.1	1.8 (0.9 - 3.3)
AA	21 (21.9)	48 (33.8)	0.5	0.8 (0.4 - 1.7)
Dominant (GA + AA vs. GG)			0.4	1.3 (0.7 - 2.4)
Recessive (AA vs. GG + GA)			0.06	0.6 (0.3 - 1)
G	95 (49.5)	130 (45.8)		
A	97 (50.5)	154 (54.2)	0.4	0.9 (0.6 - 1.2)

[a]Values are expressed as No. (%) unless otherwise indicated.

Table 3. The Frequency of Alleles and Genotypes of *COMT* rs4680 Polymorphism in the Case and Control Groups (Female Subsamples)[a]

COMT, rs4680	OA, Females, N = 9	Control, Females, N = 25	P Value	OR (95% CI)
GG	1 (11.1)	5 (20.0)		1
GA	8 (88.9)	8 (32.0)	0.2	5 (0.5 - 53)
AA	0	12 (48.0)	-	-
Dominant (GA + AA vs. GG)			0.5	2 (0.2 - 20)
Recessive (AA vs. GG + GA)			-	
G	10 (55.5)	18 (36)		
A	8 (44.5)	32 (64)	0.1	0.4 (0.2 - 1.3)

[a]Values are expressed as No. (%) unless otherwise indicated.

Table 4. The Frequency of Alleles and Genotypes of *COMT* rs4680 Polymorphism in the Case and Control Groups (Male Subsamples)[a]

COMT, rs4680	OA, Males, N = 87	Control, Males, N = 117	P Value	OR (95% CI)
GG	19 (21.8)	31 (26.5)		
GA	47 (54.0)	50 (42.7)	0.3	1.5 (0.8 - 3.1)
AA	21 (24.1)	36 (30.8)	0.9	0.9 (0.4 - 2.1)
Dominant (GA + AA vs. GG)			0.4	0.3 (0.7 - 2.5)
Recessive (AA vs. GG + GA)			0.3	0.7 (0.4 - 1.3)
G	85 (48.8)	112 (47.9)		
A	89 (51.2)	122 (52.1)	0.8	1 (0.6 - 1.4)

[a]Values are expressed as No. (%) unless otherwise indicated.

subsamples) (15). Contrary to these results, Oosterhuis et al. found a marked association between rs4680 polymorphism in Hispanic women and heroin addiction, but not in men (36). Moreover, Enoch et al. demonstrated the major effect of Val^{158} on smoking in American-Indian women and suggested that there may be sex differences in the genetic origins of alcoholism and smoking in that population, overlapping in genetic vulnerability to both addictions in women (37).

This study has several limitations. First, based on the information available about the effect of rs4680 on enzyme activity, we did not evaluate the expression of the *COMT* gene. The second limitation of this study was the small sample size (especially in the female group).

5.1. Conclusions

In the current study, we found no significant correlation between rs4680 polymorphism of the *COMT* gene and opioid addiction. According to the size of the Iranian population and possible genetic differences among them, investigations in different cities can be extremely helpful for a better understanding of the association between variations of the *COMT* gene and opioid addiction in Iran. On the

other hand, due to conflicting results in studies with different ethnicities and population sizes, additional studies of the correlation between Val[158]Met polymorphism and other variants of the *COMT* gene in a larger population and in both genders with opioid and other addictions should be done to obtain more reliable results and find new potential biomarkers for drug dependence.

Footnotes

Authors' Contribution: The conception and design of the study, statistical analysis, and manuscript preparation were performed by Fatemeh Dahmardeh and collecting of data was made by Alireza Rezaeifar.

Conflict of Interests: There is no conflict of interest.

Ethical Approval: This study was approved by the Research Council and Ethics Committee of the University of Zabol, under the code IR.UOZ.REC.1393.01.

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Patient Consent: The participants provided written informed consent.

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