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Original Article

Association of catechol-O-methyltranferase 472G > A (Val158Met) polymorphism with susceptibility to fibromyalgia syndrome

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ABSTRACT

Background: Several lines of research have suggested that the 472G > A (Val158Met) polymorphism at Catechol-O-methyltranferase (COMT) gene is implicated in the pathophysiology of FMS. Here, we have evaluated the association of COMT 472G > A polymorphism with risk of FMS.
Methods: In this study 250 patients with FMS and 250 healthy controls were evaluated for COMT 472G > A polymorphism by RFLP-PCR assay.
Results: There were no significant differences in the allele and genotype frequencies of COMT 472G > A polymorphism between FMS cases and healthy controls.
Conclusions: Our results suggested that the COMT 472G > A polymorphism may not be risk factor for development of FMS.

1. Introduction

Fibromyalgia, also called fibromyalgia syndrome (FMS), is a multifactorial, generalized pain disease, characterized by widespread musculoskeletal pain.[1,2] The major symptoms of FMS can severely impact everyday activities. The symptoms of FMS are modulated by certain factors e.g., weather, physical activity, physical or mental stress, and sleep quality. National Fibromyalgia Association (NFA) has estimated that FMS affects around 10 million people in the U.S. and an estimated 3–6% of the world population.[3] A review based of previous studies estimated the prevalence of FMS in the general population between 0.2 and 6.6%, in women between 2.4 and 6.8%, in urban areas between 0.7 and 11.4%, in rural areas between 0.1 and 5.2%.[4] FMS is a central nervous system malfunction with genetic and environmental factors being involved in the etiology of the disease,[5–7] while its pathogenesis is only poorly understood.[8]

Genome-wide association studies (GWAS) using population-based designs and linkage studies have identified many genetic markers associated with susceptibility to FMS.[9–11] The association between polymorphism in the Catechol-O-methyltransferase (COMT) gene and the risk of FMS has been studied previously.[1,10] COMT is one of the several enzymes that degrade catecholamines, catecholestrogens, and various drugs and substances with a catechol structure. Further, COMT catalyzes the transfer of a methyl group from S-adenosylmethionine to catecholamines.[12,13] Human COMT gene locus is mapped to chromosome 22q11.2, spans 28 kb, and contains 6 exons of with exons 1 and 2 being noncoding.[12] Several polymorphisms have been reported at COMT gene, while COMT 472G > A (Val158Met, rs4680) polymorphism is the most studied polymorphism in different disorders.[12] The COMT 472G > A polymorphism is located in exon 4 at nucleotide 472 and leads to amino acid substitution at codon 158 (Val158Met).[14] This functional polymorphism is associated with three to four-fold decrease in methylation activity of the COMT enzyme and distinguishes the COMT-H (high activity) and COMT-L (low activity) alleles. Thus, it is

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biologically reasonable to hypothesize that individuals who carry the COMT-Met allele are more likely to experience different diseases.[15,16]

In recent years, many studies have focused on COMT 472G > A polymorphism implication in FMS across different ethnicities.[6,17,18] Nevertheless, their results have been inconsistent and a unified conclusion is yet to be reached. This inconsistency may result from small sample sizes, varied ethnicities, and use of different genotyping methods. Therefore, more studies with a larger sample size are required. Therefore, we conducted this case control study to evaluate the association between COMT 472G > A polymorphism and risk of FMS.

2. Materials and methods

2.1. Study population

With all criteria based on the Declaration of Helsinki, this study was permitted by the Ethics and Research Committee. A written informed consent was obtained from all cases and controls. A total of 250 patients diagnosed with FMS were recruited from March 2013 and July 2018. Patients with systemic, inflammatory, infectious, and neurologic diseases or other chronic disorders were excluded. Diagnosis of FMS was based on clinical symptoms (myalgic score), laboratory markers, and imaging examination (MRI). Patients meeting the criteria of the American College of Rheumatology (ACR) for FMS were selected. The control group was comprised of 250 matched (age and gender) healthy subjects free from personal or family history of FMS, migraine and chronic pain. All FMS cases and controls were older than 40 years (Table 1).

2.2. Genotyping

Peripheral blood was collected by venipuncture from participants into EDTA tube for DNA isolation. Genomic DNA was extracted from 200 μL of whole blood using a commercial DNA Blood Mini Kit (QIAGEN, Tehran, Iran) according to the manufacturer's instructions. DNA concentration was measured by spectrophotometry (NanoDrop 2000, Thermo Scientific) diluted to nearly 40 ng/μL. The COMT 472G > A polymorphism information was obtained from NCBI website. COMT 472G > A polymorphism was genotyped by restriction fragment length polymorphism (PCR-RFLP) assay as previously described.[19–22] The primers of COMT 472G > A (rs4680) polymorphism were as follows: forward 5′-CGAGGCTCATCACCATCGAGATC-3′ and reverse 5′-CTGACAACGGGTCAGGAATGCA-3′. PCR was performed in a reaction volume of 20 μL containing 100–150 ng genomic DNA, 10 μM of each primer, 2.3 mM MgCl2, 200 μM of each dNTP, 1X Taq buffer (10 mM Tric-HCl pH 8.4, 50 mM KCl), and 0.70 units of Taq DNA polymerase. PCR amplification was performed with an initial denaturation step at 94 °C for 5 min followed by 35 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 1 min, and a final prolonged step of 5 min at 72 °C. The PCR products were digested using NlaIII enzyme restriction enzyme at 30 °C for 2 h. Then, the digested products were separated on 2% agarose gel, stained with ethidium bromide, and visualized on UV transilluminator. Based on the results, homozygotes for COMT158Met generated fragments of 72 and 36 bp, heterozygotes presented 108, 72 and 36 bp fragments, and homozygotes for COMT158Val generated only a 108 bp fragment.

Table 1
Characteristics of patients with FMS and control groups.

Variables	FMS (n = 230)	Control (n = 230)	P-Value
Age(Year)			
Mean (± SD)	42.11 ± 9.21	43.13 ± 8.23	0.053
Range	40–61	40–59	
Gender			0.805
Male	38(15.2)	40(16.0)	
Female	212(84.8)	210(84.0)	
BMI (± SD)	26.43 ± 4.17	26.65 ± 5.13	0.498

NA: Not Applicable.

2.3. Statistical analysis

The statistical analysis was performed using the statistical software SPSS20.0 (IBM SPSS Statistics 20). Continuous variables were expressed as mean ± SD or median (minimum-maximum) and categorical variables as frequency and percentage. Pearson Chi-square test was used to determine the differences between groups for categorical variables. The observed genotype frequencies in healthy controls were tested for deviation from Hardy-Weinberg equilibrium (HWE) via the Chi-square goodness-of-fit test. Odds ratios (OR) and 95% confidence intervals (CI) were calculated to assess the association between COMT 472G > A polymorphism and susceptibility to FMS. All the P values < 0.05 were considered to be statistically significant.

3. Results

The characteristics of the two groups of 250 FMS patients and 250 control subjects are shown in Table 1. The mean ages of the FMS patients and controls were 58.3 ± 9.6 and 59.5 ± 8.9 years, respectively. There was no significant difference in the gender mean age and BMI between cases and controls, indicating subjective matching (Table 1).

The allele and genotype frequencies of the COMT 472G > A polymorphism are reported in Table 2. The observed genotype frequencies of polymorphism in the controls are in agreement with the Hardy-Weinberg principle (p = 0.313). Further, the minor allele frequency (MAF) of the FMS group (0.458) was higher compared with that of the control group (0.434).

As shown in Table 2, the frequencies of COMT 472G > A polymorphism GG, GA, and AA genotypes in patients with FMS were 32.0%, 44.4%, and 23.6%, respectively, which were similar to those in the healthy subjects (33.6%, 46.0% and 20.4%, respectively). The frequency of mutant allele (A) in FMS patients and controls was 45.8% and 43.4%, respectively. There were no significant differences in the genotype (p = 0.687) and allele (p = 0.445) frequencies of COMT 472G > A polymorphism between cases and controls (Table 2). Table 3 shows the logistic regression analysis of associations between COMT 472G > A polymorphism and FMS. The analysis showed that the A allele (OR = 1.030, 95% CI = 0.737–1.438, p = 0.865) and AA genotype (OR = 1.099, 95% CI = 0.718–1.683, p = 0.664) of COMT 472G > A polymorphism were not significantly associated with increased susceptibility to FMS in the Iranian population (Table 3).

4. Discussion

FMS is a multifactorial disease caused by the interaction between multiple biological and social factors including environmental stressors,

Table 2
Genotype and allele frequencies of COMT 472G > A polymorphism in patients with FMS and controls.

Polymorphism	FMS (n = 250)		Controls (n = 250)		P-Value
	N	%	N	%	
Genotypes					0.687
GG	80	32.0	84	33.6	
GA	111	44.4	115	46.0	
AA	59	23.6	51	20.4	
Alleles					0.445
G	271	54.2	283	56.6	
A	229	45.8	217	43.4	

Table 3
Logistic regression analysis of associations between COMT 472G > A polymorphism and FMS.

Polymorphism	FMS (n = 250)	Controls (n = 250)	Odds Ratio		
			OR	90% CI	P-Value
Genotypes					
GG	80(32.0)	84(33.6)	Ref.		
GA	111(44.4)	115(46.0)	0.937	0.659–1.333	0.719
AA	59(23.6)	51(20.4)	1.205	0.789–1.842	0.388
Alleles					
G	271(54.2)	283(56.6)	Ref.		
A	229(45.8)	217(43.4)	1.102	0.859–1.414	0.445
Genetic Mode					
Dominant	170(68.0)	166(66.4)	1.075	0.740–1.562	0.703
Recessive	191(76.4)	199(79.6)	0.830	0.543–1.263	0.388

OR: Odds Ratio; CI: Confidence Interval.

psychosocial variables, and genetic factors.[23,24] Mechanisms to explain the association between genetic variants at COMT gene and susceptibility to FMS remain controversial. It is suggested that reduced COMT activity leads to elevated levels of catecholamines, such as epinephrine or norepinephrine, promoting pain production by stimulation of β2-adrenergic receptors in the peripheral and central nervous systems.[25,26] Thus, exploring conclusive evidence of the association between COMT 472G > A polymorphism and the risk of FMS is clinically significant.

To date, several studies have evaluated the association between COMT 472G > A polymorphism and susceptibility to FMS.[23,24] Those studies' findings suggest that the Met/Met genotype (AA) is associated with reduced COMT enzyme activity and increased risk of behavioral and emotional disturbances (such as impulsiveness). Our results showed that the frequency of the mutant allele of COMT 472G > A polymorphism was higher in FMS patients than in healthy controls. However, there was no significant association between COMT 472G > A polymorphism and increased risk of FMS in the Iranian population. Note that the sample size of our study was slightly small which might limit the statistical significance of our findings. Similarly, consistent with our findings, Estévez-López et al. found no association between COMT 472G > A and FMS, either susceptibility or pain in southern Spanish women.[27] Likewise, in a case-control study of 112 FMS patients and 110 controls, Barbosa et al. failed to show a significant association between the COMT 472G > A polymorphism and with increased risk of FMS in the Brazilian population. However, they reported that the COMT 472G > A polymorphism might be associated with severity of pain in the FMS patients.[28] On the other hand, our findings were not in line with some previous studies on FMS. In 2015, Lee et al. performed a meta-analysis based on ten case-control studies with a total of 993 patients with FMS and 778 healthy controls. They found that the COMT 472G > A polymorphism was significantly associated with susceptibility to the FMS 23. In the most recently published case-control study, Park et al. reported that the COMT 472G > A polymorphism was associated with increased risk of FMS in a Korean population.[18] Inanir et al. in a case-control study, reported that the COMT 472G > A polymorphism was positively associated with FMS development, which may be implicated the clinical symptoms of FMS in the Turkish population 5. Also, in a study based on a Spanish population, Martínez-Jauand et al. evaluated the association between rs6269, rs4633, rs4818, and rs4680 polymorphisms at COMT gene and development of FMS. They found that the Val158Met polymorphism plays a key role in pain sensitivity in FMS patients.[29] In 2012, Desmeules et al. reported that there was a significant association between COMT 472G > A polymorphism and psychological distress.[30] In addition, they found that COMT 472G > A polymorphism may be a useful marker for identifying FMS subgroups and thus the diagnosis and treatment of FMS.

5. Conclusion

Our results suggested that the COMT 472G > A polymorphism was not significantly associated with increased risk of FMS in the Iranian population. Further large-scale and well-designed studies are required to validate our findings in the future.

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