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ORIGINAL ARTICLE

ASSOCIATION BETWEEN THE *CATECHOL-O-METHYLTRANSFERASE* Val158Met POLYMORPHISM WITH SUSCEPTIBILITY AND SEVERITY OF CARPAL TUNNEL SYNDROME

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ABSTRACT

Carpal tunnel syndrome (CTS) is the most common entrapment neuropathy of the upper extremity. In this study, we aimed to clarify the relationships between the *catechol-O-methyltransferase* (*COMT*) gene *Val158Met* (rs4680) polymorphism and development, functional and clinical status of CTS. Ninety-five women with electro diagnostically confirmed CTS and 95 healthy controls were enrolled in the study. The functional and clinical status of the patients was measured by the Turkish version of the Boston Questionnaire and intensity of pain related to the past 2 weeks was evaluated on a visual analog scale (VAS). The *Val158Met* polymorphism was determined using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), method. We divided patients according to the genotypes of the *Val158Met* polymorphism as Val/Val, Val/Met and Met/Met. There were not any significant differences in terms of *Val158Met* polymorphisms between patients and healthy controls (p >0.05). We also did not find any relationships between the *Val158Met* polymorphism and functional and clinical status of CTS (p >0.05). In conclusion, although we did not find any relationships between CTS and the *Val158Met* polymorphism, we could not generalize this result to the general population. Future studies are warranted to conclude precise associations.

Keywords: Boston questionnaire, Carpal tunnel syndrome (CTS), *catechol-O-methyltransferase* (*COMT*) gene, pain, *Val158Met* (rs4680) polymorphism.

INTRODUCTION

Carpal tunnel syndrome (CTS) is the most common entrapment neuropathy of the upper extremity [1]. Patients with CTS may have different pain sensations. [2]. There is rising interest in the genetic predisposition to the painful conditions as they may be helpful in explaining the different pain responses to the same painful stimuli [3]. Three single nucleotide polymorphisms (SNPs) were accepted to impact pain perception: catechol-O-methyltransferase (*COMT*) *Val158Met* (rs4680), brain derived neurotrophic factor *Val66Met* and μ-opioid receptor 1 *A118G* [4,5].

The COMT is an enzyme that metabolizes catecholamines such as dopamine, norepinephrine or epinephrine and has been reported to participate in the pathogenesis of several neuropsychiatric disorders [6]. The *COMT* gene is one of the several genes taking part in nociceptive processing; however, its role remains controversial. The *COMT* gene *Val158Met* SNP leads to a substitution of valine with methionine at codon 158 on chromosome 22q11. This substitution results in differences in the COMT enzyme ac-

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tivity [7]. The presence of the valine allele results in high enzymatic activity, whereas the presence of the methionine allele is linked to low enzymatic activity [8]. The Met/Met genotype was linked to increased pain sensitivity because of low enzymatic activity that leads the accumulation of catecholamines, whereas the Val/Val genotype results in reduced pain sensitivity. In only one study was the *COMT* gene *Val158Met* SNP found not to be related to CTS development but was associated with increased perception of pain and higher disability scores [9]. However, this result is not conclusive. Therefore, we aimed to determine the associations between the *COMT* gene *Val158Met* SNP and clinical and functional status of CTS.

MATERIALS AND METHODS

Participants. Ninety-five patients with CTS and 95 age- and ethnicity-matched healthy controls were enrolled in this study. All the participants were women and housewives. Informed consent from the all participants were obtained before being admitted to the study. The study was approved by the local ethics committee.

Patients were excluded from this study if they had any of the following: having previously undergone surgery for CTS, any sensory or motor deficit in the ulnar nerve, multiple diagnosis of the upper extremities such as lateral epicondylitis or cervical radiculopathy, history of systemic disease that causes CTS such as diabetes mellitus or hypothyroidism, concomitant systemic musculoskeletal conditions such as rheumatoid arthritis or fibromyalgia, pregnancy, previous fracture of the bones of upper extremities, trauma of the neck, shoulder or upper extremities and any other neurologic diseases. The patients had to have at least four of the following to be enrolled to undergo electroneurography (ENG): pain and paresthesia in the median nerve distribution without extra median nerve territory symptoms for at least six months; increasing symptoms at night; positive Tinel sign; positive Phalen sign and self-reported hand strength deficits. The age, gender, body mass index (BMI), symptoms duration and dominant hand of the patients were recorded. Tinel and Phalen signs were noted as positive or negative.

Functional and clinical status linked to CTS was evaluated by the Turkish version of the Boston Questionnaire that consists of the symptom severity scale (SSS) and the functional status scale (FSS). The SSS and the FSS include 11 and eight questions, respectively, which are scored with one (mildest) to five (most severe) points. The overall score is the mean of 11 scores assessing pain severity, numbness and weakness at night and day and eight scores assessing the difficulty in performing common hand-related tasks. Higher scores indicate worse symptoms or dysfunction [10]. The intensity of hand or wrist pain in the last 2 weeks was rated on VAS as cm by the patients.

Electroneurography (ENG) was performed with a two-channel ENG system (Micromed S.p.A., Mogliano Veneto TV, Italy) by an experienced physician when the patient was sitting with her arm semi-flexed. Electroneurography comprised motor and antidromic sensory conduction velocities of the median and ulnar nerves. Severity of the ENG was also classified according to standardized guidelines of the American Association of Electrodiagnosis, the American Academy of Neurology and the American Academy of Physical Medicine and Rehabilitation [11]. If median nerve sensory conduction velocity is less than 40 mm/s and median nerve distal motor latency is greater than 4.20 ms, they were considered to be abnormal. Only patients with abnormal segmental comparative tests were classified to have minimal CTS, while individuals with abnormal median nerve sensory velocity conduction and normal distal motor latency were considered to have mild CTS. To have moderate CTS, ENG has to reveal both abnormal median nerve sensory velocity conduction and distal motor latency. The patients who have abnormal median nerve motor distal latency and do not have median nerve sensory response are accepted to have severe CTS. Sensory and motor nerve conduction studies of the ulnar nerve were performed in order to rule out ulnar nerve lesions.

Blood samples from both patient and control groups were collected in vacutainers containing EDTA as anticoagulant. We isolated DNA according to the procedures of the DNA isolation kit used (Gentra Puregene Blood Kit; Qiagen GmbH, Hilden, Germany) and samples were stored at –20 °C until analyzed by polymerase chain reaction (PCR).

The *COMT* gene *Val158Met* SNP was determined using the PCR-RFLP (restriction fragment length polymorphism) method. For the *COMT* gene *Val158Met* SNP, forward 5'-CTC ATC ACC ATC GAG ATC AA-3' and reverse 5'-CCA GGT CTG ACA ACG GGT CA-3' primers were used [12]. The PCR primers for *Val158Met* were used to generate a

109 bp PCR product containing the polymorphic sites. Polymerase chain reaction products were digested overnight with *Nla*III at 37 °C and analyzed on 4.0% agarose gels. The fragments used to discriminate each genotype were as follows: valine homozygotes (86 and 23 bp), Val/Met heterozygotes (86, 68, 23 and 18 bp), and methionine homozygotes (68 and 18 bp).

Statistical Analysis. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) software version 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Mean and standard deviations (mean ± SD) were used for the presentation of continuous quantitative variables. Frequencies and percentages were used for categorical data. The one-way analysis of variance (ANOVA) test was used for parametric variables, and for non parametric variables, the Kruskal-Wallis test was used for comparisons among the three groups. For evaluation of categorical variables, a χ^2 test and if needed, Fisher's exact *t*-test were used. Comparisons of genotype distribution and allele frequency between the groups were performed using a χ^2 test. To determine whether the allele frequencies were stable within patients and controls, χ^2 analysis of the Hardy-Weinberg equilibrium for the genotypes was conducted. A p value of <0.05 was accepted as significant for all statistical analyses.

RESULTS

The ages of the patients and the healthy controls were 46.3 ± 12.0 and 46.8 ± 11.3 years, respectively ($p = 0.775$). No one showed minimal CTS, 47 women had mild CTS, 40 of the patients had moderate CTS, and the remaining eight had severe CTS. The mean duration of the symptoms was 31.5 ± 36.6 months, while the mean pain level relating to the last 2 weeks was 7.01 ± 3.4 cm on VAS.

The *Val158Met* genotype distributions in women with CTS and healthy women significantly deviated from the Hardy-Weinberg equilibrium (p <0.001 for both). There was not a significant difference in the distribution of the genotypes and alleles of the *COMT* gene *Val158Met* SNP between women with CTS and healthy women ($p = 0.46$) (Table 1). We also did not find any significant difference according to genotypes in terms of symptom duration, severity of pain and ENG, and Boston FSS and SSS (Table 2).

Table 1. Distribution of the *Val158Met* genotypes and alleles of the *catechol-O-methyltransferase* gene in women with carpal tunnel syndrome and healthy women.

Parameters	Women with CTS (n = 95)	Healthy Controls (n = 95)	p Value
Val158Met Polymorphism Genotype			
Met/Met	5 (5.3%)	2 (2.1%)	
Val/Met	60 (63.2%)	65 (68.4%)	0.460
Val/Val	30 (31.6%)	28 (29.5%)	
Alleles			
Met	70 (41.9%)	69 (41.9%)	0.915
Val	120 (58.1%)	121 (58.1%)	

Val: valine; Met: methionine; CTS: carpal tunnel syndrome.

Table 2. Demographic and clinic features in women with the carpal tunnel syndrome according to the *Val158Met* polymorphism genotypes.

Parameters	Met/Met	Val/Met	Val/Val	p Value
Age (years)	40.7±12.3	47.0±11.6	46.3±11.5	0.418
BMI (kg/cm^2)	27.5±5.6	29.6±4.8	29.4±5.5	0.312
Symptom duration (months)	54.0±84.2	28.5±23.0	33.8±46.5	0.303
Severity of pain (cm)	8.0±2.7	7.4±3.2	6.3±4.0	0.392
Severity of ENG (mild/moderate/severe)	2/2/1	26/28/6	19/10/1	0.347
Tinel sign (N/P/PP)	0/4/3	3/21/14	2/35/13	0.572
Phalen sign (N/P/PP)	0/5/3	3/20/14	2/35/13	0.551
Boston FSS	1.8±0.9	2.5±3.0	2.1±1.0	0.530
Boston SSS	2.8±1.4	2.9±0.9	2.8±0.9	0.986

CTS: carpal tunnel syndrome; Met: methionine; Val: valine; BMI: body mass index; ENG: electroneurography; N: bilateral negative; P: unilateral positive; PP: bilateral positive; Boston FSS: Boston functional status scale; Boston SSS: Boston symptom severity scale.

DISCUSSION

Lower *COMT* gene activity that is linked to Met/Met genotype, results in elevated levels of catecholamines, which stimulate β2 adrenergic receptors in the peripheral and central nervous system [13,14]. The clinical status in CTS is generally thought be a result of the entrapment of the median nerve, nevertheless, current results revealed the association of the Met/Met genotype with higher pain sensitivity and disability in a number of chronic musculoskeletal disorders such as fibromyalgia [15], temporomandibular pain [16] and CTS [9]. However, our data suggested that there was no relationship between pain severity and disability and the *COMT* gene *Val158Met* SNP in the patients with CTS, similar to some previous studies investigating chronic musculoskeletal disorders [17] and neuropathic [18] and widespread pain [19]. These discrepancies may be due to the fact that a patient's SNP in a potential candidate gene does not act alone, but in interaction with environmental and ethnic factors to reveal personal manifestation in the disease course.

The *COMT* gene *Val158Met* SNP Met/Met genotype was found to be related with higher anxiety, depression and disability in fibromyalgia [20,21]. The *COMT* gene SNPs related to pain sensitivity such as *Val158Met* was found to be related with severity of fibromyalgia in Spanish patients, whereas there was no relation in Mexican patients [22]. The patients with the Met/Met genotype had worse clinical features in long-lasting low-back pain, sciatica and lumbar disc herniation [23,24]. Nevertheless, the exact role of the *COMT* gene *Val158Met* SNP remains controversial. Supporting all these results: gender, ethnicity and psychological state were accepted to interact with the genes in pain responses to painful conditions [5].

Similar to a recent study, we did not find any relationships between the *COMT* gene *Val158Met* SNP and development of CTS [9]. The patient population in the latter study also consisted of solely female patients similar to the present study.

There are several limitations in the present study. The population in this study includes only female patients and the participants were all from a secondary hospital. These conditions may limit the results to the general population. Moreover, only one SNP of the *COMT* gene was investigated in this study; therefore, we could not exclude the other genetic influences.

In conclusion, we found no relationships between the *Val158Met* SNP, CTS and clinical outcomes. Genetic researches can help to offer special care to the patients; however, the results of the reports investigating the relationships between the *COMT* gene *Val158Met* SNP and pain perception in painful conditions are conflicting. New studies with a greater number of candidate genes to CTS and pain, in a larger population including both male and female patients from several hospitals and geographic regions, should be designed to establish precise relations.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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