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RESEARCH ARTICLE

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The investigation of association between *IL-1Ra* and *ACE* I/D polymorphisms in carpal tunnel syndrome

Betul Cevik[1] | **Akin Tekcan**[2] | **Ahmet Inanir**[3,4] | **Semiha Gulsum Kurt**[1] | **Serbulent Yigit**[5]

[1]Faculty of Medicine, Department of Neurology, Gaziosmanpasa University, Tokat, Turkey

[2]Faculty of Medicine, Department of Medical Biology, Ahi Evran University, Kirsehir, Turkey

[3]Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Gaziosmanpasa University, Tokat, Turkey

[4]Academic Pain-Hernia Center, Samsun, Turkey

[5]Faculty of Medicine, Department of Medical Biology, Gaziosmanpasa University, Tokat, Turkey

Correspondence
Betul Cevik, Faculty of Medicine, Department of Neurology, Gaziosmanpasa University, Tokat, Turkey.
Email: cevikbetul78@hotmail.com

Background: Carpal tunnel syndrome (CTS) is a common neurologic impairment caused by injury on the median nerve in the wrist, characterized by pain and loss of sensory. CTS usually occurs through three factors, such as a mechanical pressure on median nerve, immunologic changes, and oxidative stress. The aim of this study was to evaluate the influence of interleukin-1 receptor antagonist (*IL-1Ra*) and angiotensin-converting enzyme (*ACE*) I/D polymorphisms on the susceptibility of patients to the CTS.

Methods: One hundred fifty-eight patients with CTS and 151 healthy controls were enrolled in this study. Each patient was analyzed according to diseases symptoms, such as gender, a positive Tinel's sign, a positive Phalen maneuver, disease sides, EMG findings, and clinical stage. We applied the polymerase chain reaction (PCR) to determine the polymorphisms of *IL-1Ra* and *ACE* I/D.

Results: The statistically significant relation was not found between *IL-1Ra*, *ACE* I/D polymorphisms and CTS (respectively, $P>.05$; $P>.05$, OR: 1.51, CI: 0.82-1.61). Additionally, in the result of the statistical analysis compared with gene polymorphisms and clinical characteristics, we did not find any correlation ($P>.05$).

Conclusions: Our findings showed that there are no associations of *IL-1Ra* and *ACE* I/D polymorphisms with susceptibility of a person for the development of CTS. So, it means that these polymorphisms do not create a risk for the development of CTS. Further studies with larger populations will be required to confirm these findings in different study populations.

KEYWORDS

angiotensin-converting enzyme, carpal tunnel syndrome, entrapment neuropathy, interleukin-1 receptor antagonist, polymorphism, susceptibility

1 | INTRODUCTION

Carpal tunnel syndrome (CTS) is a common neurologic impairment caused by injury of the median nerve in the wrist, characterized by pain, weakness, paresthesia, and loss of sensory.[1-3] In a general population, the prevalence of CTS ranges between 2.7% and 5.8%. Additionally, CTS is occurring six and four times more frequently in patients with type 1 and 2 diabetes according to the general population, respectively.[4] The CTS may occur after long-term repetitive hand and wrist movements because of the median nerve entrapment, which leads to impaired neural microcirculation. These situations show an important role for environmental factors in the development of CTS.[3] Several theories have been proposed to explain this phenomenon including trauma, infections, and autonomic factors.[1,2] Its underlying general pathophysiology is ascribed to a pressure on the peripheral nerves in the carpal tunnel.[5] However, the CTS may show a family attribute, indicating a potential genetic contribution to this condition.[6] A new pathogenetic theory based on the immunologic characteristics.[7,8] Also, the CTS has been connected with systemic amyloidosis caused by amyloid accumulation within the nerve and synovial tissue of the

flexor tendons (secondary CTS). The possible genetic factors responsible for the development of CTS are unknown,[3] and the molecular mechanisms of the CTS are still unclear.

The response mediated by these cytokines may be of particular relevance within peripheral nerves subjected to localized compression in the CTS, because of the lack of autoregulation of the endoneurial vascular bed.[4] Increased vascular permeability that is associated with increased cytokine expression is also present after the nerve entrapment.[9] CTS affects the fingertips innervated by the median nerve.[1] The expression is that the imbalances of the immunostimulating and immunosuppressive neuropeptides release in the median nerve are the causes for the various dermatologic symptoms. According to this opinion, the variability of dermatologic symptoms in CTS depends on the prevalence of both immunosuppressive and immunostimulating neuropeptides such as interleukin (IL).[8,10] The altered expression of several cytokines, such as interleukin-1β (IL-1β) and interleukin-6 (IL-6), and growth factors such as vascular endothelial growth factor (VEGF) in tendon and ligament injuries has been reported.[11-13] The expression of VEGF, VEGFR-1, and VEGFR-2 was increased in patients with CTS.[4] Interleukin-1 (IL-1) is one of the key modulators of the inflammatory response, and its activity is regulated by its receptor antagonist, interleukin-1 receptor antagonist (IL-1Ra), an anti-inflammatory cytokine.[14] Therefore, the investigation of *IL-1R* gene mutations and *ACE* gene mutations associated with the endothelial damage and oxidative stress may give important results. To assess whether the *IL-1Ra* and *ACE* genes are the genes affecting the predispose to CTS, we investigated the differential distribution of genotypes and alleles in relation to clinical characteristics, such as sex, age at the onset and EMG findings, and clinical stage of the CTS.

2 | MATERIALS AND METHODS

2.1 | Participants

One hundred fifty-eight patients (patient group, 136 females and 22 males), with carpal tunnel syndrome and one hundred fifty-one healthy participants (control group, 116 female, and 35 male) without any reported history of CTS symptoms or surgery, were recruited for this study from the Physical Therapy and Rehabilitation and Neurology clinics in the Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey. The controls and patients with CTS were matched for the type of occupation and years of exposure for the wrist activity.

Before participation in this study, the participants were informed about the procedures and they gave written informed consent (according to the Declaration of Helsinki). In addition, each participant completed a questionnaire containing personal details as well as self-reported personal and family medical history. Each patient was analyzed according to disease symptoms, such as gender, a positive Tinel's sign, a positive Phalen maneuver, disease sides, EMG findings, and clinical stage. This study was approved by the Ethics Committee of the Faculty of Medicine, Gaziosmanpasa University (14-KAEK-162).

2.2 | DNA extraction and genotype determinations

Genomic DNA was isolated from the whole venous blood samples using a commercial DNA isolation kit (Sigma-Aldrich, Taufkirchen, Germany). The samples were preserved in EDTA tubes and stored at −20°C. The *IL-1Ra* exon 2 polymorphism was analyzed using the following primers: forward 5′-CTCAGCAACACTCCTAT-3′ and reverse 5′-CCTGGTCTGCAGGTAA-3′. Genomic DNA was amplified in a final volume of 30 μL, containing 10 mmol/L TRIS pH 8.3, 50 mmol/L KCl, 1.5 mmol/L $MgCl_2$, 200 μmol/L each dNTP, 1 μmol/L of each primer and 2 U Taq polymerase. The polymerase chain reaction (PCR) conditions consisted of the initial denaturation at 95°C was followed by 35 cycles of denaturation at 94°C, annealing at 60°C, extension at 72°C, 1 minute each and a final extension at 72°C for 7 minutes. The PCR products were analyzed by 2% agarose gel electrophoresis. The alleles were identified as: allele 1 (4 repeats) 410 bp, allele 2 (2 repeats) 240 bp, allele 3 (3 repeats) 325 bp, allele 4 (5 repeats) 500 bp, and allele 5 (6 repeats) 595 bp as described earlier.[15]

ACE gene I/D polymorphism (rs1799752) genotypes were determined using PCR using the primers and conditions described earlier.[4] Reactions were performed with 10 pmol of each primer: forward 5′-CTGGAGACCACTCCCATCCTTTCT-3′, and reverse 5′-GATGTGGC CATCACATTCGTCAAGT-3′. Amplification was performed in a thermal cycler for 30 cycles with denaturation at 94°C for 40 seconds, annealing at 56°C for 40 seconds and extension at 72°C for 40 seconds, followed by a final extension at 72°C for 10 minutes. PCR products were analyzed on 2% agarose gels after staining with ethidium bromide and were visualized using an ultraviolet transilluminator. The polymorphisms detected by the PCR were evident as an approximately 490 bp fragment in the presence of the insertion (I) allele and as an approximately 190 bp fragment in the absence of insertion (D) allele. In heterozygous samples, two bands (490 and 190 bp) were detected as described previously.[16] To confirm the accuracy and reproducibility of this method, each PCR reaction included internal controls for each genotype. Second PCR analysis was performed to confirm the samples, but the results of the samples were not clear.

2.3 | Predictions of protein-protein interactions

The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) online database was used to retrieve the functional partners of IL1Ra and ACE proteins. The STRING analyses were performed according to some parameters of the IL1Ra and ACE proteins, such as neighborhood, fusion, occurrence co expression, experiments, database, and text mining. Additionally, predictions with a confidence score higher than 0.7 were included in this study.

2.4 | Statistical analysis

All statistical analyses of the data were performed using the computer software SPSS version 15.0 (IBM Corp.; Armonk, NY, USA) for Windows and OpenEpi Info software package program (Atlanta, GA,

USA, http://www.openepi.com). Genotype distribution of the genotype and allele frequency of the *IL-1Ra* and *ACE* genes between patients with CTS and controls were compared using Chi-Square test. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. Hardy-Weinberg equation was applied to the genotypes of the patients and controls. *P* values .05 or less were considered statistically significant.

3 | RESULTS

Demographic variables and baseline characteristics of patients are presented in Table 1. The mean age±standard deviation (SD) was 48.21±11.22 years in patients and 45.79±11.02 years in control group. There were 22 (13.9%) males, 136 (86.1) females and 35 (23.2%) males, 116 (76.8%) females in patient and control groups, respectively. The statistically significant relationship was not found between *IL-1Ra* polymorphism and CTS (*P*>.05; Table 2). On the other hand, there was no statistically significant relationship between *ACE* I/D polymorphism and the CTS (*P*>.05, 1.51 (0.82-1.61; Table 3). Also, in the result of the statistical analysis compared with gene polymorphisms and clinical characteristics, such as gender, a positive Tinel's sign, a positive Phalen maneuver, disease sides (left, right, and bilateral), EMG findings, and clinical stage, there was no significantcorrelation.

STRING database annotates the functional interactions among the proteins in a cell. Analyzing the IL1Ra and ACE proteins with STRING database, predicted the functional partners of two proteins with high confidence (score: 0.7) were found as follows: HNRNPD, IL1R1, IL1R2, IL1A, IL1B, IL1RAP, TOLLIP, MYD88, IRAK2, IRAK3, and SOD3, MDK, ACE2, TFPI, AGT, REN, ATP6AP2, KNG1, NOS3, and BDKRB2, respectively. The interaction network of these proteins is shown in Figure 1A and B. Also, in the results of protein-protein interaction analysis, it was seen that none of the partner proteins of IL1Ra and ACE were associated with the carpal tunnel syndrome as reported in the literature.

TABLE 1 Clinical and demographic factors of controls and patients with CTS

Characteristic	Control group	Study group
Gender, male/female, n (%)	35/116 (23.2/76.8)	22/136 (13.9/86.1)
Age, mean±SD, y	45.79±11.02	48.21±11.22
Height, mean±SD, y	163.53±6.09	162.55±4.79
Weight, mean±SD, y	76.98±8.67	73.44±8.93
BMI, mean±SD, y	28.49±3.12	27.77±3.09
Age onset of disease	—	43.94±11.28
Dominant hand		
Left/Right (%)	—	14.2/85.8
Time of diagnosis, y	—	1.93±1.25
Diseases duration	—	3.02±1.85
Family history		
neg/pos (%)	—	80.4/19.6
Disease side		
Left/Right/Bilateral (%)	—	10.8/10.8/78.4
Tinel's sign		
neg/pos (%)	—	25.7/74.3
Phalen maneuver		
neg/pos (%)	—	40.5/59.5
EMG findings		
Normal (%)	—	23
Slightly symptoms (%)	—	25.7
Mid symptoms (%)	—	45.3
Severe symptoms (%)	—	6
Clinical stage		
No symptoms (%)	—	4.7
Nocturnal paresthesia (%)	—	30.4
Diurnal paresthesia (%)	—	47.3
Loss of sensation (%)	—	14.9
Atrophy, plegy, Thenar muscle power loss, Motor loss (%)	—	2.7

BMI, body mass index; CTS, carpal tunnel syndrome; EMG, electromyography; SD, standard deviation; neg/pos, negative/positive.

4 | DISCUSSION

Carpal tunnel syndrome is a common peripheral neuropathy.[17] And, because the CTS considerably decreases the quality of life of the people, it is required that the underlying causes of CTS should be defined. In the analysis of the literature, it is seen that there are different ideas such as a mechanical pressure in the wrist on the median nerve,[5] immunologic changes,[7,8] and oxidative stress[18] are involved in the development of CTS. It has certainly proven that the mechanical pressure on the median nerve in the wrist is one of the important causes for the CTS symptoms.[5] Immunologic changes are associated with the factors that are influencing the neuropeptide release as secondary compression.[2] We conducted this study to assess whether any relationship between the *IL-1Ra*, *ACE* genes and susceptibility to the CTS exists or not, and found that there were no statistically significant association between *IL-1Ra*, *ACE* I/D polymorphisms and CTS susceptibility of patients.

IL-1 family is a group of 11 cytokines, which induces a complex network of pro-inflammatory cytokines, regulates, and initiates inflammatory responses. IL-1Ra regulates both IL-1α and IL-1β pro-inflammatory activity by competing with them for binding sites of the receptor.[19] Burger et al.[10] showed that although the functional *IL-1β*, *IL-6*, and *VEGFA* variants were not associated, the *IL-6R* rs2228145 (A/C) polymorphism was associated with the increased CTS risk, and they expressed that IL gene family variants can affect each other through gene-gene interactions within the cytokine signaling cascades and may increase the risk of CTS. In other study, the absence of local inflammation was determined by measuring the gene expression level of pro-inflammatory cytokines

TABLE 2 Genotype and allele frequencies of IL-1Ra VNTR gene polymorphisms in patients with CTS and control groups

	Genotypes											
IL1Ra	1.1 (n)	1.2 (n)	1.3 (n)	1.4 (n)	1.5 (n)	2.2 (n)	2.3 (n)	2.4 (n)	3.3 (n)	4.4 (n)	4.5 (n)	*P*
Patients (n:158)	77	56	1	10	0	13	0	0	0	0	1	>.05
Controls (n:151)	80	42	2	7	1	13	1	2	1	1	1	

	Alleles				
	1 IL-1Ra 1 (±)	IL-1Ra 2 (±)	IL-1Ra 3 (±)	IL-1Ra 4 (±)	IL-1Ra 5 (±)
Patients	221/95 (69.9/30)	82/234 (25.9/76.4)	1/315 (0.3/99.6)	11/305 (3.4/96.5)	1/315 (0.3/99.6)
Controls	212/90 (70.1/29.8)	71/231 (23.5/76.4)	5/297 (1.6/98.3)	12/290 (3.9/96)	2/300 (0.6/99.3)
P	>.05	>.05	>.05	>.05	>.05
OR (CI 95%)	0.98 (0.69-1.39)	1.14 (0.79-1.64)	0.18 (0.0-1.37)	0.87 (0.36-2.04)	0.47 (0.01-6.29)

CI, confidence interval; CTS, carpal tunnel syndrome; IL-1Ra, interleukin-1 receptor antagonist; OR, Odds ratio.

TABLE 3 Genotype and allele frequencies of ACE gene polymorphisms in patients with CTS and control groups

Gene	CTS patients n=158	Controls n=151	*P*	OR (CI 95%)
ACE (I/D)				
Genotypes				
DD	77 (48.73%)	65 (43.04%)	>.05	
ID	64 (40.50%)	69 (45.69%)		
II	17 (10.75%)	17 (11.25%)		
Alleles				
D	218 (68.98%)	199 (65.89%)	>.05	1.51 (0.82-1.61)
I	98 (31.01%)	103 (34.10%)		

ACE, angiotensin-converting enzyme; CI, confidence interval; CTS, carpal tunnel syndrome; OR, Odds ratio.

(interleukin-1 β, -17, and -6 and tumor necrosis factor a) in carpal ligament.[3] Freeland et al.[20] analyzed the levels of IL-1 between patients with CTS and controls and showed no difference in the serum and flexor tenosynovial consistently in our study. Additionally, *IL-1*β rs16944 and *IL-6* rs1800795 interact with a *COL5A1* variant rs12722 to modulate the risk of chronic Achilles tendinopathy.[11] The C-allele of *IL-1*β rs16944 causes increased expression of IL-1β mRNA which in turn increases extracellular matrix degradation,[11,21] and is therefore proposed to be associated with the increased risk of tendinopathy.[10] It has also been hypothesized that tendinopathy might be mediated through the cytokines involved in signal transduction.[10,11] Also, Barker et al.[22] demonstrated that IL-1β is an early mediator of upregulation of nerve growth factor. In the light of literature, we think that the variations in IL gene family may have an effect to susceptibility to CTS through gene-gene interactions. Sud and Freeland[23] expressed that the levels of IL-6 are significantly higher in the flexor tenosynovium of patients with CTS but are normal in the serum, while IL-1 levels are normal in both the serum and the tenosynovium of patients with CTS. So, IL-6 stimulates the production of acute-phase proteins and IL-1 is associated with the activation of T-cells as well as inducing growth factors and inflammatory mediations.

ACE activity was strongly influenced by the human *ACE* gene polymorphism,[24] and angiotensin II increases under the effect of ACE and plays a role on tissue damage through proinflammatory and pro-fibrotic effects. The changes in the tissue levels of angiotensin II were associated with the neural ischemia in especially patients with diabetic peripheral neuropathy. Also, it is stated that angiotensin II may affect the endothelial damage and oxidative stress.[25] When it is considered, the role of the oxidative stress in the development of CTS,[18] we think that the *ACE*

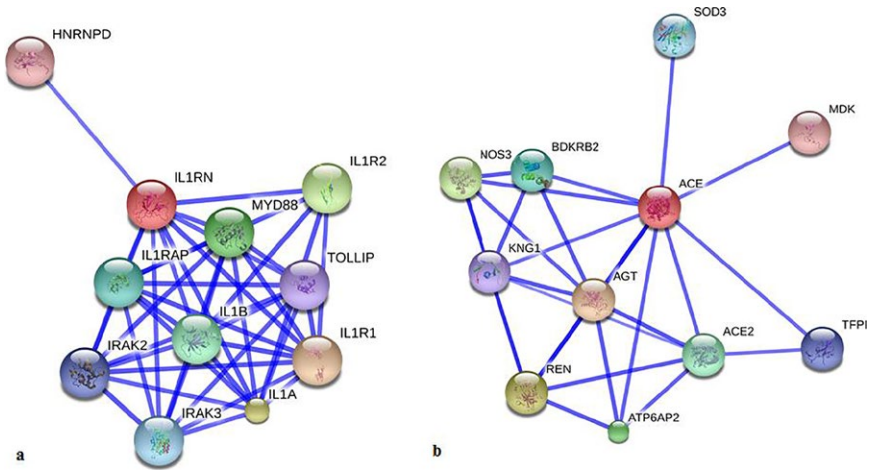

FIGURE 1 (A) IL1Ra (IL1RN) and (B) ACE protein-protein interactions with its partners revealed after STRING analysis

polymorphisms should be analyzed in patients with CTS . In the present study, statistically significant relationship between Turkish patients with CTS and controls in terms of the frequencies of genotype and alleles of *ACE* I/D polymorphism is not found. The *ACE* I/D polymorphism are associated with different risks for developing diabetic complications such as neuropathy.[26] It is stated that there is a change in the oxidative stress and antioxidant defences in patients with CTS. Also, the increased total oxidative stress and decreased total antioxidant status might stimulate fibrosis through disturbed signaling pattern in the tenosynovium and median nerve.[27] Angiotensin II has been shown to increase sympathetic nerve activity and oxidative stress in the circulatory system.[28] Additionally, it is suggested that the angiotensin II may affect endothelial damage and oxidative stress.[25,29] It was seen that none of the partner proteins of IL1Ra and ACE were associated with carpal tunnel syndrome in the literature.

Present study includes several limitations: the sample size was small, which may result in false-positive and false-negative results in the genetic association studies by decreasing the statistical power of the study. Moreover, it is another limitation that serum and/or flexor tenosynovial IL-1 and ACE levels were not measured in patients and control groups.

In conclusion, our findings showed that there are no associations of *IL-1Ra* and *ACE* I/D polymorphisms with susceptibility of a person for the development of CTS. Also, in the analysis of the results compared with clinical characteristics, such as clinical stage, EMG findings, and disease side, it was determined that there was no association between *IL-1Ra* and *ACE* I/D polymorphisms and clinical characteristics of CTS. Indeed, we think that the interactions of IL gene family members should be investigated of their roles in the susceptibility of patients to the CTS.

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