The Effect of IL-4 Gene Polymorphism in Carpal Tunnel Syndrome

Karpal Tünel Sendromunda IL-4 Gen Polimorfizminin Etkisi

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Özet

Amaç: Karpal tünel sendromu el bileğinde medyan sinirin disfonksiyonu ve/veya lezyonları nedeniyle elde uyuşma ve ağrı ile karakterize nörolojik bir hastalıktır. KTS'nin patogenezi tam olarak açık olmamakla birlikte, genetik faktörler KTS'ye yatkınlıkta rol oynayabilmektedir. Sitokinler gibi biyokimyasal faktörler KTS'nin neden olduğu nöropatide rol alabilmektedir. Bu çalışmanın amacı bir Türk populasyonunda KTS yatkınlığı ile IL-4 VNTR polimorfizmi arasındaki ilişkileri ve IL-4 VNTR polimorfizminin KTS'li hastalarda hastalığın gelişimi üzerine etkilerini incelemektir. Gereç ve Yntem: Çalışmaya KTS'li 155 hasta ve 140 sağlıklı kontrol dâhil edilmiştir. Hasta ve kontrollerin genomik DNA'lar izole edilmiş ve IL-4 geni 70 bç'lik VNTR polimorfizmleri polimeraz zincir reaksiyonu kullanılarak genotiplenmiştir. Bulgular: Hasta ve kontrol grupları arasında IL-4 genotip dağılımı açısından istatistiksel olarak anlamlı bir fark bulunmamaktadır (p>0.05). Ancak, P1 allelinin KTS'li hastalarda sağlıklı kontrollere göre anlamlı derecede fazla olduğu saptanmıştır (p<0.05; OR: 1.608, 95% CI: 1.05-2.44). Ayrıca, hastaların klinik özellikleri ile IL-4 genotip dağılımı arasında herhangi bir farklılık bulunmadığı belirlenmiştir. Tartışma: Bizim bulgularımız Türk populasyonunda IL-4 VNTR polimorfizminin KTS ile ilişkili bir belirteç olmadığı ve P1 allelinin ise KTS ile ilişkili olabileceğini işaret etmektedir. KTS'de IL-4 ve anti-inflamatuar sitokinlerin etkilerinin tespit edilmesi için daha büyük hasta populasyonu ile daha ileri çalışmaların yapılmasına ihtiyaç bulunmaktadır.

Anahtar Kelimeler

Karpal Tünel Sendromu, Sitokinler, İnterlökin-4, Yatkınlık

Abstract

Aim: Carpal tunnel syndrome (CTS) is a neurological disorder characterized by paresthesia and pain in the hands due to lesions and /or dysfunction of the median nerve at the wrist. The exact pathogenesis of CTS is not clear. Genetic factors may play a role in CTS susceptibility. Biochemical mediators such as cytokines may have a role in carpal tunnel mediated neuropathy. The aim of the present study was to analyse the association of IL-4 VNTR polymorphism with CTS susceptibility and disease progression in patients with CTS in a Turkish population. Material and Method: The study included 155 patients with CTS and 140 healthy controls. Genomic DNA was isolated and genotyped using polymerase chain reaction (PCR) for the IL-4 gene 70 bp VNTR polymorphisms. Results: There was no statistically significant difference between the groups with respect to IL-4 genotype distribution (p>0.05). The P1 allele was significantly higher in CTS patients than in healthy controls (p<0.05; OR: 1.608, 95% CI: 1.05-2.44). There was no difference between IL-4 genotype distribution and clinical characteristics of patients (p>0.05). Discussion: Our findings indicate that the IL-4 70 bp VNTR polymorphism is not a relevant CTS marker and that the P1 allele may be related to CTS in a Turkish population. Further research with larger patient populations is necessary to ascertain the implications of IL-4 and anti-inflammatory cytokines polymorphisms in CTS.

Keywords

Carpal Tunnel Syndrome, Cytokines, Interleukine-4, Susceptibility

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Introduction

Carpal tunnel syndrome (CTS) is a neurological disorder characterized by paresthesia and pain in the hands due to lesions and /or dysfunction of the median nerve at the wrist [1-4]. The carpal tunnel is located at the base of the palm. CTS is defined as "a symptomatic compression neuropathy of the median nerve at the level of the wrist" by the American Academy of Orthopaedic Surgeons (AAOS) [5]. In the general population, CTS has been found to have a prevalence rate of 3.8% and an incidence rate of 1.8/1,000 [6,7] with more frequency in women than in men; the prevalence rate is 9.2% in women and 6% in men. [5]. CTS is one of the most common upper limb compression neuropathies. CTS accounts for approximately 90% of all entrapment neuropathies. It is due to an entrapment of the median nerve in the carpal tunnel at the wrist [6]. Also, CTS is the most common neuropathy during pregnancy [8].

CTS is associated with compression, some anomalies, autoimmune or hematologic disorders, arthritis, trauma, or neoplasms [9]. The exact pathogenesis of CTS is not clear. There are several theories which have been put forward to explain the symptoms. The mechanical compression, micro-vascular insufficiency, and vibration theories are the most popular ones. According to the mechanical compression theory, symptoms of CTS are due to compression of the median nerve in the carpal tunnel. The micro-vascular insufficiency theory proposes that the lack of blood supply leads to depletion of nutrients and oxygen to the nerve, causing it to slowly lose its ability to transmit nerve impulses. According to the vibration theory, the symptoms of CTS could be due to the effects of long-term use of vibrating tools on the median nerve in the carpal tunnel [10]. Also, some research indicates that biochemical mediators, including the free oxygen radicals prostoglandin E2 (PGE2) and interleukin-6 (IL-6), may ultimately be involved in the pathophysiology of idiopathic CTS by cause of ischemia [11]. While the nature of CTS has been extensively studied, little is known about the genetic background of this disease. Several authors have suggested that genetic factors are associated with CTS [12,13]. The investigations performed to determine the genetic background of CTS focused on proinflammatory cytokines such as IL-1, IL-6 and tumour necrosis factor α (TNF- α). These cytokines are released by peripheral nervous system cells including macrophages, T-cells, and Schwann cells in response to inflammation, tissue injury, and immunological reactions. Subsequently, they initiate a molecular cascade to activate other proinflammatory cytokines and growth factors that contribute to neuropathic pain [14]. They can also directly modulate neuronal hypersensitivity and elicit spontaneous neuronal discharges [15]. While the pro-inflammatory cytokines such as IL-6 and IL-18 have the pronociceptive effect, the anti-inflammatory cytokines such as IL-4 or IL-10 play an important role in nociception, thereby inhibiting the development of neuropathic pain [16]. Additionally, evaluation of nociception was used to monitor the functional recovery of the injured nerve and the nociception increased after median nerve compression [17]. Therefore, it is important to investigate associations between CTS and anti-inflammatory cytokines such as IL-4 that have a role in neuronal hypersensitivity and the nociception process. However, until now, there has been no report about the relationship of IL-4 gene intron 3 VNTR polymorphism and CTS. The aim of the present study is to analyze the association of IL-4 VNTR polymorphism with CTS susceptibility and disease progression in patients with CTS in a Turkish population.

Materials and Methods

Subjects

One hundred fifty-five patients (patient group, 135 females and 20 males) with carpal tunnel syndrome and 140 healthy participants (control group, 106 female and 34 male, without any reported history of CTS symptoms or surgery), were recruited for this study from Physical Therapy and Rehabilitation and the Neurology clinics in the Faculty of Medicine, Gaziosmanpasa University, Tokat, Turkey. The controls and the patients with CTS were matched for type of occupation and years of exposure to wrist activity (Table 1). Prior to participation in this study, the participants were informed about the procedures and gave written informed consent (according to the Declaration of Helsinki). In addition, a questionnaire containing personal details and self-reported personal and family medical history

Table 1. Clinical and demographics features of controls and patients with CTS

Characteristic	Control group	Study group
Gender, male/female, n (%)	34/106 (24.3/75.7)	20/135 (12.9/87.1)
Age, mean ± SD, years	46.59±11.03	46.85±11.45
Height, mean ± SD, years	163.53±6.09	162.55±4.79
Weight, mean ± SD, years	76.98±8.67	73.44±8.93
BMI, mean ± SD, years	28.49±3.12	27.77±3.09
Age onset of disease	--	47.31±11.90
Dominant hand		
Left/Right (%)	--	14.2/85.8
Time of diagnosis, years	--	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, EMG: Electromyography

questionnaires were completed by each participant. This study was approved by the Ethics Committee of the Faculty of Medicine, Gaziosmanpasa University (14-KAEK-039).

Genotyping

DNA was extracted from 2 mL venous blood according to kit procedure (Sigma, USA) and stored at -20°C. To detect 70 bp VNTR polymorphism of IL-4 gene PCR assay as described by Mout et al. [18] was used. PCR was performed with a 25 µl reaction mixture containing 50 ng DNA, 0.8 lM of each primer, 200 lM of each dNTP, 2.5 mM MgCl2, 0.5 U Taq polymerase, 109 KCl buffer (MBI, Fermentas). Amplification was carried out using primers F5' AGGCTGAAAGGGGGAAAGC-3', R 5'-CTG TTCACCT-CAACTGCTCC-3' with initial denaturation at 95ºC for 5 min, 30 cycles of denaturation at 94ºC for 30 s, annealing at 58ºC for 45 s, extension at 72ºC for 1 min and final extension at 72ºC for 10 min. The PCR products were visualized on 3% agarose gel stained with ethidium bromide. PCR product was of 183 bp for P1 allele and 253 bp for P2 allele. In order to validate the accuracy and reproducibility of this method, each PCR reaction included internal controls for each genotype. A second PCR was performed to confirm samples in which results were not clear. Also, to confirm the accuracy of the genotyping, repeated analysis was performed on randomly selected samples. No discrepancies were found.

Statistical Analysis

Analysis of the data was performed using the computer software SPSS 16.0 (SPSS, Chicago, IL, USA) and OpenEpi Info software package program. Continuous data were given as mean ± SD (standard deviation) and (min–max). The frequencies of the alleles and genotypes in patients and controls were compared with x2 analysis. Odds ratio (OR) and 95% confidence intervals (CIs) were calculated. p value smaller than 0.05 (two-tailed) was regarded as statistically significant. Power analysis was made by using Minitab 15.0 package program. Hardy-Weinberg equilibrium was assessed by Chi-square analysis.

Results

Demographic variables and baseline characteristics of patients are given in Table 1. The mean age ± standard deviation (SD) was 46.85±11.45 years in patients and 46.59±11.03 years in the control group. There were 135 females (87.1) and 20 males (12.9) and 106 female (75.7) and 34 male (24.3) in patient and control groups, respectively. Table 2 presents the distribution of IL-4 70 bp VNTR mutation profiles in patients and control groups. There was no statistically significant difference in the genotype frequencies at IL-4 70 bp VNTR gene (p>0.05), but allele frequencies showed a statistically significant association (p=0.02). Also, many clinical characteristics (gender, age, height, weight, BMI, age onset of disease, dominant hand, time of diagnosis, family history, disease side, Tinel's sign, Phalen maneuver, EMG findings, and clinical stage) were analyzed. Among the demographic and clinical characteristic of FM patients, no statistically significant difference was found between carriers and non-carriers of IL-4 70 bp VNTR polymorphism (Table 1 and 3). It was concluded that IL-4 70 bp VNTR polymorphism may cause FM especially in P1 allele form.

Discussion

The studies related to carpal tunnel genetic background and future therapeutic strategies are focused on cytokines and proteoglycans [19,20]. It has been reported that proinflammatory cytokines play an important role in the pathophysiology of neuropathic pain syndromes, CTS, and herniated intervertebral disks [21]. Interleukin-6 (IL-6) is a multifunctional bioactive cytokine whose actions include the modulation of proliferation and differentiation. Also, IL-6 has a role in the development, differentiation, regeneration, and degeneration of neurons in the peripheral and central nervous systems [11,15].

Table 2. Genotype and allele frequencies of IL-4 70 bp VNTR gene polymorphisms in CTS patient and control groups

Gene	CTS patients n=155	Controls n=140	p	OR (CI 95%)
IL-4 (70 bp VNTR)				
Genotypes				
P1/P1	10 (6.5 %)	5 (3.6 %)	0.09	
P1/P2	50 (32.3 %)	33 (23.6 %)		
P2/P2	95 (61.3 %)	102 (72.9 %)		
Alleles				
P1	70 (22.5 %)	43 (15.3 %)	0.02	1.608 (1.05-2.44)
P2	240 (77.4 %)	237 (84.6 %)		

The results that are statistically significant are typed in bold

Table 3. IL-4 genotype frequencies according to clinical characteristics in CTS patients

Clinical Characteristics		IL-4zgenotypes			p
		P1P1	P1P2	P2P2	
Gender	Male	1	8	11	>0.05
	Female	10	42	84	
Tinel's sign	Negative	2	14	22	>0.05
	Positive	4	33	73	
Phalen maneuver	Negative	2	21	37	>0.05
	Positive	4	26	58	
Disease sides	Left	1	7	8	>0.05
	Right	2	5	9	
	Bilateral	3	35	78	
EMG findings	Normal	2	15	17	>0.05
	Slightly symptoms	2	7	29	
	Mid symptoms	2	22	43	
	Severe symptoms	0	3	6	
Clinical Stage	No symptoms	0	2	5	>0.05
	Nocturnal paresthesia	3	12	30	
	Diurnal paresthesia	2	22	46	
	Loss of sensation	1	10	11	
	Atrophy, plegy, thenar muscle power loss, motor loss	0	1	3	
Age onset of disease		40.83±8.97	46.59±1.17	48.07±1.20	>0.05

In other studies related to the genetics of carpal tunnel syndrome, it has been reported that proteasome modulator 9 gene creates a risk for the development of carpal tunnel syndrome [22]. Lupsi et al showed that Y169H missense variant segregates with an axonal neuropathy, whereas the nonsense R954X mutation is associated with subclinical evidence of carpal tunnel syndrome; therefore, haploinsufficiency of SH3 domain and tetratricopeptide repeats-containing protein 2 may cause susceptibility to carpal tunnel syndrome [23]. These data suggest that multiple cytokines, including IL-1 and IL-6, produced from tenosynovial tissues in patients with dialysis-associated amyloidosis might induce the proliferation of synovial cells and might cause carpal tunnel syndrome [24]. Penas et al reported that the Val158Met polymorphism Catechol-O-methyltransferase that inactive of the catecholamine neurotransmitters seems not to be a risk factor for the development of CTS [2]. In previous studies, the fibrotic factors such as transforming growth factor-beta, connective tissue growth factor, type 1 collagen, and type 3 collagen are effective in CTS susceptibility [25]. Additionally, Burger et al showed that sequence variants of the COL5A1 3□-untranslated region (UTR) are associated with altered risk of CTS [12].

In the present study, we analysed the frequencies of 70 bp VNTR polymorphisms at intron 3 of anti-inflammatory cytokine IL-4 in CTS patients in a Turkish population. Our results show that while there was no statistical significance between the groups with respect to IL-4 genotype distribution ($p>0.05$), the allele frequencies of the patients and healthy controls are statistically significant ($p<0.05$). The main findings of this study are that the percentages of IL-4 70 bp VNTR polymorphism alleles are significantly different between patients and controls. We think that it is important to investigate associations between CTS and anti-inflammatory cytokines such as IL-4 that have a role in neuronal hypersensitivity and nociception process.

Conclusion

These findings show that there is an association of IL-4 gene 70 bp VNTR polymorphism P1 allele with susceptibility of a person for the development of carpal tunnel syndrome ($p<0.05$). The results of this study are important because this is the first report that investigates the relationships between susceptibility to carpal tunnel syndrome and IL-4 gene intron 3 VNTR polymorphisms. Additional analyses with larger populations are required to confirm these findings in different study populations.

Competing interests

The authors declare that they have no competing interests.

References

1. Patijn J, Vallejo R, Janssen M, Huygen F, Lataster A, van Kleef M et al. Carpal tunnel syndrome. Pain Pract 2011;11(3):297-301.
2. Fernández-de-las-Peñas C, Ambite-Quesada S, Ortega-Santiago R, Martínez-Perez A, Díaz HF, Martínez-Martín J et al. Catechol-O-methyltransferase Val-158Met polymorphism is associated with pain and disability, but not widespread pressure pain sensitivity, in women with carpal Tunnel syndrome. Pain Physician 2013;16(5):591-600.
3. Zieske L, Ebersole GC, Davidge K, Fox I, Mackinnon SE. Revision carpal tunnel surgery: a 10-year review of intraoperative findings and outcomes. J Hand Surg Am 2013;38(8):1530-9.
4. Kim PT, Lee HJ, Kim TG, Jeon IH. Current approaches for carpal tunnel syndrome. Clin Orthop Surg 2014;6(3):253-7.
5. Ibrahim I, Khan WS, Goddard N, Smitham P. Carpal tunnel syndrome: a review of the recent literature. Open Orthop J 2012;6:69-76.
6. Aroori S, Spence RA. Carpal tunnel syndrome. Ulster Med J 2008;77(1):6-17.
7. Ghasemi-Rad M, Nosair E, Vegh A, Mohammadi A, Akkad A, Lesha E et al. A handy review of carpal tunnel syndrome: From anatomy to diagnosis and treatment. World J Radiol 2014;6(6):284-300.
8. Massey EW, Guidon AC. Peripheral neuropathies in pregnancy. Continuum (Minneap Minn) 2014;20(1):100-14.
9. Yang TH, Thoreson AR, Gingery A, An KN, Larson DR, Zhao C et al. Collagen gel contraction as a measure of fibroblast function in carpal tunnel syndrome. J Biomed Mater Res A 2015;103(2):574-80.
10. Viikari-Juntura E, Silverstein B. Role of physical load factors in carpal tunnel syndrome. Scand J Work Environ Health 1999 25(3):163-85.
11. Freeland AE, Tucci MA, Barbieri RA, Angel MF, Nick TG. Biochemical evaluation of serum and flexor tenosynovium in carpal tunnel syndrome. Microsurgery 2002;22(8):378-85.
12. Elstner M, Bettecken T, Wasner M, Anneser F, Dichgans M, Meitinger T et al. Familial carpal tunnel syndrome: further evidence for a genetic contribution. Clin Genet 2006;69(2):179-82.
13. Burger MC, De Wet H, Collins M. The BGN and ACAN genes and carpal tunnel syndrome. Gene 2014;551(2):160-6.
14. Bolin LM, Verity AN, Silver JE, Shooter EM, Abrams JS. Interleukin-6 production by Schwann cells and induction in sciatic nerve injury. J Neurochem 1995;64:850-8.
15. Frieboes LR, Palispis WA, Gupta R. Nerve compression activates selective nociceptive pathways and upregulates peripheral sodium channel expression in Schwann cells. J Orthop Res 2010;28(6):753-61.
16. Rojewska E, Popiolek-Barczyk K, Jurga AM, Makuch W, Przewlocka B, Mika J. Involvement of pro- and antinociceptive factors in minocycline analgesia in rat neuropathic pain model. J Neuroimmunol 2014;277(1-2):57-66.
17. Marcioli MA, Coradini JG, Kunz RI, Ribeiro Lde F, Brancalhão RM, Bertolini GR. Nociceptive and histomorphometric evaluation of neural mobilization in experimental injury of the median nerve. Scientific World Journal 2013;476890:1-6
18. Mout R, Willemze R, Landegent JE. Repeat polymorphisms in the interleukin-4 gene (IL4). Nucleic Acids Res 1991;19(13):3763.
19. Bianchi E, Taurone S, Bardella L, Signore A, Pompili E, Sessa V. Involvement of pro-inflammatory cytokines and growth factors in the pathogenesis of Dupuytren's contracture: a novel target for a possible future therapeutic strategy? Clin Sci (Lond) 2015;129(8):711-20.
20. Burger MC, de Wet H, Collins M. Interleukin and growth factor gene variants and risk of carpal tunnel syndrome. Gene 2015;564(1):67-72.
21. Kraychete DC, Sakata RK, Issy AM, Bacellar O, Jesus RS, Carvalho EM. Proinflammatory cytokines in patients with neuropathic pain treated with Tramadol. Rev Bras Anestesiol 2009;59(3):297-303.
22. Gragnoli C. Proteasome modulator 9 and carpal tunnel syndrome. Diabetes Res Clin Pract 2011;94(2):47-9.
23. Lupski JR, Reid JG, Gonzaga-Jauregui C, Rio Deiros D, Chen DC, Nazareth L et al. Whole-genome sequencing in a patient with Charcot-Marie-Tooth neuropathy. N Engl J Med 2010;362(13):1181-91.
24. Miyasaka N, Sato K, Kitano Y, Higaki M, Nishioka K, Ohta K. Aberrant cytokine production from tenosynovium in dialysis associated amyloidosis. Ann Rheum Dis 1992;51(6):797-802.
25. Gingery A, Yang TH, Passe SM, An KN, Zhao C, Amadio PC. TGF-β signaling regulates fibrotic expression and activity in carpal tunnel syndrome. J Orthop Res 2014;32(11):1444-50.

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