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Is Complement Factor H Tyr402His Variant a Potential Cause of Ankylosing Spondylitis?

Kompleman Faktör H Tyr402His Varyant Ankilozan Spondilitin Olası Bir Nedeni Olabilir mi?

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Abstract

Aim: Ankylosing spondylitis (AS) is an autoimmune disease caused by chronic inflammatory response. Complement system is the major component of the innate immune defence. In this study, we investigated the potential association between complement factor H *(CFH)* gene Tyr402His variant (rs1061170) with AS in a Turkish population.

Methods: Seventy-eight AS patients and 80 healthy individuals were enrolled in the present study as case and control subjects, respectively. The Tyr402His variant of *CFH* gene was analysed by PCR-RFLP method.

Results: There was no statistically significant difference between AS patients and healthy controls in terms of *CFH* Tyr402His genotype and allele frequencies. However, the visual analogue scale (VAS) daytime and the AS Quality of Life (ASQoL) were significantly different according to *CFH* Tyr402His genotype distribution (p=0.032 and p=0.036, respectively). VAS of daytime and ASQoL were higher in subjects carrying Tyr402His variant Tyr/Tyr + Tyr/His genotypes compared to those carrying His/His genotype.

Conclusion: This is the first study evaluating the association between *CFH* Tyr402His and susceptibility to AS in a Turkish population. Although *CFH* Tyr402His variant was not considered a candidate gene for AS susceptibility in our samples, some clinical findings seem to be associated with genotype distribution of *CFH* Tyr402His variant.

Keywords: Ankylosing spondylitis, complement factor H, variant

Öz

Amaç: Ankilozan spondilit (AS) kronik enflamatuar cevabın neden olduğu otoimmün bir hastalıktır. Kompleman sistemi doğal bağışıklığın esas savunma sistemidir. Bu çalışmada, Türk toplumunda kompleman faktör H *(CFH)* geni Tyr402His varyantı (rs1061170) ve AS arasındaki olası ilişkiyi araştırdık.

Yöntemler: Bu çalışmaya 78 AS hastası ve 80 sağlıklı birey, olgu ve kontrol bireyleri olarak alındı. *CFH* Tyr402His varyantı PPCR-RFLP yöntemi ile analiz edildi.

Bulgular: AS hastaları ve sağlıklı kontroller arasında *CFH* Tyr402His genotip ve alel sıklıkları açısından istatistiksel olarak önemli fark yoktu. Ancak, gündüz vizüel analog skala (VAS) ve AS Yaşam Kalitesi Ölçeği (ASQoL) *CFH* Tyr402His genotip dağılımına göre önemli derecede farklıydı (p=0.032, p=0.036, sırasıyla). Gündüz VAS ve ASQol Tyr402His varyantı Tyr/Tyr + Tyr/His genotiplerini taşıyan kişilerde His/His genotipi taşıyanlara göre daha yüksekti.

Sonuç: Bu, Türk toplumunda *CFH* Tyr402His varyantı ve AS yatkınlığı arasındaki ilişkiyi değerlendiren ilk çalışmadır. *CFH* Tyr402His varyantı bizim örneklerde AS yatkınlığı için aday bir gen olmasa da, bazı klinik bulgular *CFH* Tyr402His varyantın genotip dağılımı ile ilişkili görünmektedir.

Anahtar Sözcükler: Ankilozan spondilit, kompleman faktör H, varyant

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Introduction

Ankylosing spondylitis (AS) is a chronic, progressive autoimmune illness that involves the axial and sacroiliac joints. The majority of patients with AS eventually manifest spine malformations, resulting in functional impairment (1). The prevalence of AS ranges between 0.1% and 1.4% worldwide, and it is seen more frequently in Eurasia (2). AS occurs more frequently in males, with a male/female ratio of 2:1 (3). Similar to other autoimmune diseases, the pathogenesis of AS remains unclear. Genetic and environmental factors may play a role in the etiology. Previous studies reported that major histocompatibility alleles, particularly HLA-B27, may account for upto one-third of the genetic effect. (4), hence suggesting that there could be other susceptible genes that play significant roles in the onset of this disease. Dysregulation or overactivation of the immune system appears to be crucial since several studies demonstrated several immune cells, secreted-mediators, and markers that are involved in the pathogenesis of AS (5).

The complement system plays a key role in innate immunity which has functions varying from eliminating foreign pathogens to modulating immune responses and playing a part in the homeostasis chiefly through its cleaved products, such as pro-inflammatory C3a and C5a, opsono-cytophagic C3b/iC3b, and cytolytic membrane attack complex (MAC, with C5b-9n components) (6). The complement regulator, complement factor H (*CFH*, OMIM 134370) regulates the alternative pathway of the complement system; it has anti-inflammatory effect and protects the host tissue from damage. It has been reported that genetic variation in the *CFH* gene, which is found on 1q31.3 region of chromosome 1, is related with a higher risk of inflammatory diseases (7).

A single nucleotide polymorphism (SNP), Tyr402His, found in exon 9 of the *CFH* gene manifests a tyrosine to histidine change at amino acid position 402 in the CFH protein that modifies the complement activity (8). Complement dysfunctions, such as unregulated activation and inadequate regulation, exerts its harmful potential against host cells, implying that the complement system plays a crucial role in several human disorders, including autoimmune, inflammatory, and infectious diseases (9).

With this background, we postulated that the *CFH* gene might be a risk factor for AS. In the present study, we aimed to examine the association of CFH Tyr402His variant in patients with AS and control subjects in Turkey.

Methods

Study Population

Seventy-eight AS patients as cases were recruited from the Department of Physical Medicine and Rehabilitation at the Medical Faculty, Gaziantep University (Gaziantep, Turkey) The patients were diagnosed with AS after routine examinations, X-ray, computed tomography and nuclear magnetic resonance imaging according to the Modified New York Criteria for AS (10). Exclusion criteria were diabetes mellitus, cancer, severe liver and kidney failure, and being on therapy for any chronic inflammatory disease. Meanwhile, the control group was composed of 80 healthy individuals. The patients and control groups were matched in terms of age and ethnic background. All subjects provided written informed consent after being informed about the details of the study. The ethics committee of the Gaziantep University Ethics Committee approved the project in accordance with the tenets of the Helsinki Declaration and the National Ethical Guideline for Medical Research (no: 2016/308).

Assessment Criteria

Visual Analog scale (VAS) was applied to assess the level of pain. Pain during night time and daytime were evaluated. Disease activity was assessed by the Bath AS Disease Activity index (BASDAI) from 0 (no symptoms) to 10 maximal symptoms) on a numeric scale. Functional impairment was evaluated by the Bath AS Functional index (BASFI) from 0 to 10. Higher values of BASFI indicate poorer functional ability. To assess the quality of life, the AS Quality of Life (ASQoL) questionnaire was used.

Genotyping

DNA samples were extracted from the peripheral blood in all subjects by the salting out method (11). Then the DNA samples were stored at -20 °C. The *CFH* Tyr402His genotype was determined by using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method (12). Briefly, a 244-bp DNA fragment containing the variant site was amplified with the primer pairs of *CFH*- F (5'- ACT GTG GTC TGC GCT TTT G3') and CFR-R (5'-TTT TTG GAT GTT TAT GCA ATC TT-3'). PCR was performed in a 10-µL reaction mixture containing 25 ng DNA, 0.1 mM each primer, and 1 µ Maxima® HotStart Green PCR MasterMix (Thermo Scientific). The thermal profile consisted of an initial denaturation step 2 minutes at 94 °C, followed by 34 cycles of 30 seconds at 94 °C, 40 seconds at 60 °C, 55 seconds at 72 °C, and a final elongation step of 5 minutes at 72 °C. PCR product was digested by FastDigest *NlaIII* restriction enzyme (Thermo Scientific) at 37 °C for 5 minutes. The restriction products were separated in 2% agorose gel and visualised by ultraviolet illumination. The CFH Tyr/Tyr genotype consisted of a single 244-bp band; the 402 His/His genotype had two bands, 161-bp and 83-bp, whereas the Tyr/His heterozygous genotype had three bands: 244-bp, 161-bp, and 83-bp. Random samples were selected,

50% of experiments were repeated, and the concordance rate was 100%.

Statistical Analysis

Analysis of the data was performed using the computer software SPSS 15.0 (SPSS, Chicago, IL) and Open Epi Info software package program. Continuous data were given as mean ± SD (standard deviation) and (min-max). Differences in CFH Tyr402His genotype distribution between the patients and controls were compared with the chi-square test and, Fisher's exact test was used when needed. The Hardy-Weinberg equilibrium (HWE) was calculated using the de-finetti program (Online HWE and Association Testing-Institut für Human genetik, Munich, Germany). The association between the CFH Tyr402His variant with clinical manifestations, namely VAS (night time and daytime), BASDAI, BASFI, and ASQoL was investigated through the Mann-Whitney U test. A p value of less than 0.05 (two-tailed) was regarded as statistically significant.

Results

Genotype and allele frequencies of *CFH* Tyr402His variant are listed in Table 1. Among 78 patients and 80 healthy controls with *CFH* Tyr402His variant, Tyr/Tyr homozygote accounted for 39.7%, 40.0%, Tyr/His heterozygote accounted for 44.9%, 41.3%, and His/His genotype accounted for 15.4%, 18.7 in patients and controls, respectively. The frequency of Tyr allele was 62.18%, 60.63% and His allele was 37.82%, 39.37% in patients and controls, respectively. There was no significantly difference in distribution of either genotypes or alleles between AS patients and healthy subjects (Table 1).

Next, the association between the CFH Tyr402His variant and clinical manifestations, namely VAS (night time and daytime), BASDAI, BASFI, and ASQoL was investigated (Table 2). VAS of daytime and ASQoL were significantly different among AS patients with Tyr/Tyr + Tyr/His and His/His genotype of *CFH* Tyr402His variant (p=0.032). VAS of daytime and ASQoL were significantly increased in patients carrying Tyr/Tyr + Tyr/His genotypes in comparison with those who had His/His genotype (Table 2).

Discussion

AS belongs to the spondyloarthritis family of diseases in which certain clinical, genetic, and immunologic characteristics are common. Chronic inflammation in the joints of the vertebrae results in serious chronic pain and stiffness. This in turn leads to ankylosis, immobility and consolidation of a joint due to the disease (13). On the contrary, dysregulation or excessive activation of the immune system appears to be crucial since some researchers reported that various immune cells, secreted-

Table 1. Genotype and allele frequency of *CFH* gene Tyr402His variant between groups

CFH Tyr402His	Patients	Controls	OR	95% CI	p
Genotypes	**n=78 (%)**	**n=80 (%)**			
Tyr/Tyr	31 (39.7)	32 (40)	1.043&	0.553-1.970&	1.000&
Tyr/His	35 (44.9)	33 (41.3)	0.942*	0.471-1.884*	0.866*
His/His	12 (15.4)	15 (18.7)	1.313*	0.522-1.884*	0.562*
Alleles					
Tyr	97 (62.18)	97 (60.63)	-	-	-
His	59 (37.82)	63 (39.37)	0.937&	0.595-1.473&	0.818&

OR: Odds ratio, CI: Confidence interval, n: Number, CFH: Complement factor H
*OR (95%CI) was adjusted by age and sex, &: Fisher's Exact Test.

Table 2. Association of *CFH* Tyr402His genotypes with various clinical features of the patients

Characteristics	Tyr/Tyr + Tyr/His	His/His	p*
	n=66 (SD)	**n=12 (SD)**	
VAS of night time	5.02 (2.33)	3.92 (1.97)	0.079
VAS of day time	4.08 (2.51)	2.50 (1.73)	0.032
BASDAI	4.35 (1.85)	3.40 (1.86)	0.082
BASFI	4.05 (1.71)	2.95 (1.71)	0.053
ASQol	8.89 (4.60)	5.92 (3.45)	0.036

SD: Standard deviation, VAS: Visual Anolog scale, BASDAI: Bath Ankylosing Spondylitis Disease Activity index, BASFI: Bath Ankylosing Spondylitis Functional index, ASQol: Ankylosing Spondylitis Quality of Life, CFH: Complement factor H
*Mann-Whitney U test

mediators, and markers played an important role in the pathogenesis of AS.

The complement system is a part of the innate immunological mechanism that contains effector molecules and receptors which help in both fighting against the invasion of pathogens and regulation of the immune system. The complement cascade can be triggered by variety of molecules, such as bacterial cell-wall components and antigen-antibody complexes, leading to the activation of one of the three major complement pathways. These include classical, alternative or lectin pathways (14). The complement system contains membrane-bound regulators and receptors along with many plasma proteins that interact with several cells and mediators of the immune system (15). These interactions differ with regard to the pathophysiologic setting, and they take place at various stages of an immune reaction. Impairment in the balance of complement activation and regulation will lead to detrimental results and can contribute to several inflammatory diseases, such as age-related macular degeneration, rheumatoid arthritis (RA), systemic lupus erythematosus, and Alzheimer's disease (16-18). There are a growing number of proofs implying that the complement system influences the skeletal system (19). Herewith, the complement system modulates bone metabolism and turnover both under physiological and pathophysiological conditions. Actually, it was seen that the state of complement activation affects and modulates the development and progression of some bone-related acute and chronic inflammatory disorders (19).

The complement system has a usually well-defined effect especially in chronic inflammatory disorders, all of which are associated with extreme bone loss. Numerous complement proteins and their cleaved products have been found in synovial fluids of patients with RA, including the early complement components C1q and C4 (20), as well as pro-inflammatory cleavage products C3a (21), and C5a (22). This accumulation of complement components occurring in arthritis patients implies a mechanism of local complement generation and activation in the demarcated area of inflamed joints. For more investigation of complement involvement in arthritis, animal model trials have been conducted with a wide variety of complement-deficient or complement-co strains, most of which being based on the collagen-induced arthritis models of RA. These models virtually imitate the autoimmune and progressive features of human RA, with cartilage and bone destruction (23).

The CFH protein is a critical regulator of the alternative pathway of the complement cascade that involves the elimination of pathogens and immune complexes, and modulates adaptive immunity (24). CFH hinders complement activation by preventing the development and facilitating the decay of C3 convertase and acting as a cofactor for factor I-mediated degradation of C3b, both in plasma and on cell surfaces. Many studies showed complement activation in AS by the importantly increased complement components or activation products such as C3, C4 and C3d, and by the complement activation triggers including IgA, IgG, C-reactive protein (CRP), serum amyloid A, apolipoprotein A (25). Besides, the complement activation products including C3a and C5a may alter the expression of proinflammatory cytokines such as IL-1β, IL-6, and TNF-α in blood cells (26).

The CFH gene contains 23 exons and spans more than 94 kb of genomic DNA (27). The CFH Tyr402His variant is found in an area of CFH which binds to both heparin and C-reactive protein, and this binding could be modified by a tyrosine (Y) to histidine (H) substitution in CFH protein, leading to dysregulation of CFH (28). The adequate formation of C-reactive protein-CFH complex on cell surfaces is critical in order to reduce complement activation and diminish the secretion of the proinflammatory cytokine TNF-α (29). Previous studies reported that the *CFH* Tyr402His variant might be related to an enhanced activation of complement cascade both systemically and locally (30).

These observations led us to hypothesize that *CFH* Tyr402His variant may be involved in the pathogenesis of AS through inflammation. However, there has been no study investigating the association of the *CFH* Tyr402His variant with AS to date. We first considered the possibility that *CFH* Tyr402His variant is related to the inflammatory process in AS in a Turkish cohort. We found no evidence for the association of *CFH* Tyr402His variant with AS risk. Previous studies have demonstrated that the CFH variants were not associated with RA (31,32). Then, we compared the clinical characteristics of AS patients and genotypes of *CFH* Tyr402His. We detected a slightly significantly higher VAS of daytime and ASQoL score in subjects carrying Tyr/Tyr + Tyr/His genotypes compared to that in those with His/His genotype.

Study Limitations

There are some limitations of the present study that should be considered. Initially, we centred on only a variant involved in the pathway of CFH, other regulatory genes in the signalling pathway may also play a role in the pathogenesis of AS. Secondly, due to the relatively small sample size, the number of some homozygous variants was low in groups and thus decreased the statistical power. Finally, absence of assessment of expression levels of CFH is also a limitation of this study. The strengths of our study are its prospective nature.

Conclusion

In conclusion, this is the first research investigating the relationship between *CFH* Tyr402His genotype distribution and AS and also their association with clinical findings. Although we found no significant association between *CFH* Tyr402His variant and AS risk, our results suggest a possible association of *CFH* Tyr402His variant with clinical features including VAS and ASQoL. Genetic variants are important in AS pathogenesis, and further studies with larger populations may help control the clinical findings of patients with AS and facilitate the development of the new therapeutic agents.

Authorship Contributions

Concept: S.P., M.P., A.F.N. Design: S.P., S.G. Data Collection or Processing: M.S.A., S.G. Analysis or Interpretation: S.P., A.F.N. Literature Search: M.P., A.F.N. Writing: A.F.N.

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