
Common Crohn's disease-predisposing variants of the CARD15/NOD2 gene are not associated with Behçet's disease in Turkey

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ABSTRACT

Objective. There are many extra-intestinal findings of Crohn's disease (CD), such as oral and genital ulcers, erythema nodosum, uveitis and arthritis, resembling the manifestations of Behçet's disease (BD). It is also very difficult to distinguish the gastrointestinal involvement of BD from that of CD in some patients. Hence, this study aimed to investigate a possible involvement of the common CD-predisposing CARD15 variants in the genetic susceptibility to BD.

Methods. The study group consisted of 85 consecutive patients with BD (51 male, 34 female) of Turkish origin. Two of them had intestinal involvement. A group of 100 ethnically matched, non-related healthy volunteers were used as controls. All individuals were genotyped for 3 common CARD15 variants (R702W, G908R, and L1007fsinsC) using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method.

Results. None of the three CARD15 variants predisposing to CD was observed in patients with BD, including two patients with intestinal involvement. The R702W mutation was observed in 1 healthy chromosome, and the 3020insC mutation in 2 chromosomes. No individual was found to be homozygous or compound heterozygous for these variants.

Conclusion. These findings suggest that 3 most common CD-predisposing CARD15 variants do not constitute a genetic susceptibility factor for BD in Turkey. Further studies would be helpful to rule out a possible contribution of other rare or unknown variants and/or the effects of different ethnic backgrounds.

Introduction

Behçet's disease (BD) is a multi-system inflammatory disorder, the hall-

marks of which are recurrent oral and genital ulceration, skin lesions, and uveitis. It is now recognized to be a systemic vasculitis that also affects the joints, all types and sizes of blood vessels, the lungs, and the central nervous and gastrointestinal systems (1).

The cause of BD is not definitely known, but various immunological abnormalities induced by particular microbial agents or other environmental factors in genetically susceptible individuals have been suggested (2). The strongest evidence for a genetic susceptibility to BD is its association with a class I HLA antigen, HLA-B51. This association has been confirmed in different ethnic groups, though the role of HLA-B*51 in the pathogenesis of BD has not yet been clarified. The contribution of this HLA allele to the overall genetic susceptibility to BD is estimated to be less than 20% (3).

Spontaneous and/or induced over-expression of pro-inflammatory cytokines (mainly Th1 type) from various cellular sources seems responsible for the enhanced inflammatory reaction in BD, and it may be associated with the genetic susceptibility (2).

Caspase recruitment domain 15 (CARD15) [also, nucleotide-binding oligomerization domain 2 (NOD2)] is a member of the NOD1/Apaf1 superfamily of apoptosis regulators that is expressed in monocytes and involved in the activation of nuclear factor-κB (NF-κB), by bacterial components (4). Three major genetic alterations, R702W, G908R, and L1007fsinsC, in the CARD15 gene have been shown to increase the risk for Crohn's disease (CD) in Caucasian populations (5,6). Certain mutations in the CARD15 gene have also been implicated in Blau syndrome, a rare genetic disorder with a dominant mode of inheritance charac-

terized by the occurrence of granulomatous arthritis, uveitis, and skin lesions (7).

Both BD and CD have distinctive clinical features. However, there are many extra-intestinal findings of CD, such as oral and genital ulcers, erythema nodosum, uveitis and arthritis, resembling the manifestations of BD. It is also very difficult to distinguish the gastrointestinal involvement of BD from that of CD in some patients (8, 9). The similarities of the mucocutaneous and intestinal manifestations of both disorders can be a cause of confusion even for experienced physicians (10). Hence, this study aimed to investigate a possible involvement of the common CD-predisposing CARD15 variants in the genetic susceptibility to BD.

Patients and methods

Patients

The study group consisted of 85 consecutive BD patients of Turkish origin (51 male, 34 female) being followed at the BD Outpatient Clinic of the Division of Rheumatology. All patients met the International Study Group criteria for the classification of BD (11). Two of them had intestinal involvement, 51 had arthritis, and 44 had uveitis.

The ethical committee of the Istanbul Faculty of Medicine approved the study, and all patients gave their written consent prior to blood collection. A group of 100 ethnically matched, non-related healthy volunteers were used as controls.

Genotyping

Genomic DNA was isolated from peripheral blood leukocytes using standard procedures. Three single nucleotide polymorphisms (R702W, G908R, and L1007fsinsC) were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis method. For the determination of the missense R702W mutation (SNP 8; GenBank accession number G67950) genomic DNA was amplified with the primer pair of 5'-GTACAGGCTCCGGATGAGC-3' and 5'-CGCACAACCTTCAGATCACA-3'. The amplified 239-bp product was digested with *HpaII*. The presence of a

wild type allele resulted in four bands of 76-bp, 54-bp, 49-bp and 31-bp, whereas the profile of the R702W variant was characterized by three bands of 130-bp, 49-bp and 31-bp on ethidium bromide stained 3% agarose gel. Genotyping for the G908R (SNP 12; GenBank accession number G67951) polymorphism was carried out with PCR amplification and *HhaI* digestion as described previously by Vermeire *et al.* (12). For the L1007fsinsC frameshift mutation (SNP 13; GenBank accession number G67955), a 196-bp DNA segment was amplified with the primer pair of 5'-ACCAGACTTCCAGGATGGTG-3' and 5'-GGACAGGTGGGCTTCAGTAG-3' (13). The product was digested with *NlaIV* and electrophoresed on ethidium-bromide stained 2% agarose gel. The profile of 1007fsinsC was characterized by the presence of two bands (160-bp and 36-bp).

Statistical analysis

Genotype and phenotype frequencies for the three CARD15 gene variants were compared between BD patients and controls using a χ^2 or Fisher's exact test, as appropriate.

Results

The allele frequencies the CARD15 variants in the BD and control groups are shown in Table I. None of the three most common CARD15 alleles predisposing to CD was observed in patients with BD, including two patients with intestinal involvement. The R702W mutation was observed in 1 healthy chromosome, and 3020insC mutation in 2 chromosomes. No individual was found to be homozygous or compound heterozygous for these variants. The G908R variant was not observed among the healthy controls. No significant difference in the distribution of genotypes was observed between the BD and control groups.

Discussion

The CARD15 gene has been identified in the IBD1 locus at chromosome 16 and found to be specifically associated with CD among a group of multi-case inflammatory bowel disease families, which include families with ulcerative

Table I. Allele frequencies of the three most common Crohn's disease-associated CARD15 variants in Behçet's disease (BD) patients and healthy controls.

	BD	Controls (%)
Number of chromosomes	170	200
Variants		
R702W	0	1 (0.5)
G908R	0	0
L1007fsinsC	0	2 (1)
Overall*	0	3 (1.5)

*No homozygous and compound heterozygous individual.

colitis (5, 6). This gene functions in the sensing of intracellular bacterial peptidoglycans through the specific recognition of muramyl dipeptides and activates NF- κ B pathway (14,15). CD-predisposing CARD15 variants have been suggested to cause a defect in the recognition of colorectal luminal bacteria, which results in the activation of alternative pathways and/or a dysregulated inflammatory response (5, 15). However, Blau syndrome associated mutations in the same gene result in a peptidoglycan-independent 4-fold increase in the basal activity of the gene, which can explain the dominant mode of inheritance due to gain-of-function mutations (15).

Rahman *et al.* suggested the CARD15 as a non-MHC pleiotropic autoimmune gene, since it confers susceptibility to 2 complex (CD and psoriatic arthritis) and one Mendelian genetic disorders (Blau syndrome) (16). An increased inflammatory response to non-specific and/or various microbial stimuli is an important feature of BD. Although the relationship of HLA-B*51 with neutrophil functions is not clear, genetic factors seem to play an important role in the enhanced inflammatory reaction in BD. The increased expression of several cytokines from lymphocytes and monocytes is reported, and over-secretion of mainly Th1 type proinflammatory cytokines is prominent during the active phase of BD (2). However, increased secretion of interleukin-1 (IL-1), IL-6, TNF α and IL-8 from monocytes following lipopolysaccharide

(LPS) stimulation compared with healthy and/or disease controls can also be observed, even in inactive BD patients (2). Because of many similarities between the clinical and pathogenetic features of BD and CD, we aimed to investigate the frequency of common CD-predisposing variants in a series of BD patients. However, no CARD15 variant could be detected in this group of BD patients. This observation suggests that the common CARD15 variants do not confer susceptibility for BD. In contrast to Rahman's suggestions, these variants have not been associated with rheumatoid arthritis, ankylosing spondylitis, systemic lupus erythematosus, or sarcoidosis either (17-20).

Our study group included only 2 BD patients with gastrointestinal involvement. Therefore, it was not possible to rule out a contribution of CARD15 variants to the development of intestinal lesions in BD. It is already known that the gastrointestinal features of BD are more common in Korean and Japanese patients, and it would be necessary to investigate the CARD15 variants in these ethnic groups for clarification of this issue.

The prevalence of CARD15 polymorphisms in healthy controls and CD patients shows a variation between different ethnic groups. The carrier rate of 3% for the Turkish healthy controls is lower than most of the figures reported for European populations (5,6,12,13, 16-21). The impact of this lower frequency of the variations on the prevalence of CD and the magnitude of the CARD15 polymorphisms on the genetic susceptibility to CD in Turkey need to be explored.

It is difficult to rule out a possible role of population stratification or admixture

in the selection of the control group, as in other case-control association studies. However, the failure to identify any BD patients with the 3 most common CD-predisposing CARD15 variants suggests that these polymorphisms are not a genetic susceptibility factor for BD in Turkey. Further studies would be helpful to exclude a possible contribution of other rare or unknown polymorphisms to susceptibility to BD and/or the effects of different ethnic backgrounds with varying rates for the CARD15 variants on the gastrointestinal involvement.

References

1. SAKANE T, TAKENOM, SUZUKI N, INABAG: Behçet's disease. *N Engl J Med* 1999; 341: 1284-91.
2. GUL A: Behçet's disease: An update on the pathogenesis. *Clin Exp Rheumatol* 2001; 19 (Suppl. 24): S6-S12.
3. GULA, HAJEER AH, WORTHINGTON J, BARRATTJH, OLLIERWER, SILMAN AJ: Evidence for linkage of the HLA-B locus in Behçet's disease, obtained using the transmission disequilibrium test. *Arthritis Rheum* 2001; 44: 239-41.
4. OGURA Y, INOHARA N, BENITO A *et al.*: Nod2, a Nod1/Apaf-1 family member that is restricted to monocytes and activates NF-kappaB. *J Biol Chem* 2001; 276: 4812-8.
5. OGURA Y, BONEN DK, INOHARA N *et al.*: A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; 411: 603-6.
6. HUGOT JP, CHAMAILLARD M, ZOULALI H *et al.*: Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; 411: 599-603.
7. MICELI-RICHARD C, LESAGE S, RYBOJAD M *et al.*: CARD15 mutations in Blau syndrome. *Nat Genet* 2001; 29: 19-20.
8. KORMAN U, CANTASDEMIR M, KURUGOGLU, *et al.*: Enteroclysis findings of intestinal Behçet disease: a comparative study with Crohn disease. *Abdom Imaging* 2003; 28: 308-12.
9. LEE CR, KIM WH, CHO YS, *et al.*: Colonoscopic findings in intestinal Behçet's disease. *Inflamm Bowel Dis* 2001; 7: 243-9.
10. TUNÇ R, ULUHAN A, MELIKOGLU M, OZYAZGAN Y, OZDOĞAN H, YAZICI H: A reassessment of the International Study Group criteria for the diagnosis (classification) of Behçet's syndrome. *Clin Exp Rheumatol* 2001; 19 (Suppl.24): S45-S47.
11. INTERNATIONAL STUDY GROUP FOR BEHCET'S DISEASE: Criteria for diagnosis of Behçet's disease. *Lancet* 1990; 335: 1078-80.
12. VERMEIRE S, WILD G, KOCHER K, *et al.*: CARD15 genetic variation in a Quebec population: prevalence, genotype-phenotype relationship, and haplotype structure. *Am J Hum Genet* 2002; 71: 74-83.
13. LESAGE S, ZOULALI H, CEZARD JP, *et al.*: CARD15/NOD2 mutational analysis and genotype-phenotype correlation in 612 patients with inflammatory bowel disease. *Am J Hum Genet* 2002; 70: 845-57.
14. INOHARAN, OGURA Y, FONTALBAA, *et al.*: Host recognition of bacterial muramyl dipeptide mediated through NOD2. Implications for Crohn's disease. *J Biol Chem* 2003; 278: 5509-12.
15. CHAMAILLARD M, PHILPOTT D, GIRARDIN SE, *et al.*: Gene-environment interaction modulated by allelic heterogeneity in inflammatory diseases. *Proc Natl Acad Sci U S A* 2003; 100: 3455-60.
16. RAHMAN P, BARTLETT S, SIANNIS F, *et al.*: CARD15: a pleiotropic autoimmune gene that confers susceptibility to psoriatic arthritis. *Am J Hum Genet* 2003; 73: 677-81.
17. STEER S, FISHER SA, FIFE M, *et al.*: Development of rheumatoid arthritis is not associated with two polymorphisms in the Crohn's disease gene CARD15. *Rheumatology (Oxford)* 2003; 42: 304-7.
18. FERREIROS-VIDAL I, GARCIA-MEIJIDE J, CARREIRA P, *et al.*: The three most common CARD15 mutations associated with Crohn's disease and the chromosome 16 susceptibility locus for systemic lupus erythematosus. *Rheumatology (Oxford)* 2003; 42: 570-4.
19. VAN DER PAARDT M, CRUSIUS JB, DE KONING MH, *et al.*: CARD15 gene mutations are not associated with ankylosing spondylitis. *Genes Immun* 2003; 4: 77-8.
20. MARTIN TM, DOYLE TM, SMITH JR, DINULESCU D, RUST K, ROSENBAUM JT: Uveitis in patients with sarcoidosis is not associated with mutations in NOD2 (CARD15). *Am J Ophthalmol* 2003; 136: 933-5.
21. TUKEK T, SHALATA A, PRESENT D, *et al.*: Crohn disease: Frequency and nature of CARD15 mutations in Ashkenazi and Sephardi/Oriental Jewish families. *Am J Hum Genet* 2004; 74: 623-36.