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Is there a genetic predisposition for Turkish patients with sarcoidosis in the 329-bp region containing the *BTNL2* rs2076530 polymorphism?

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Background/aim: Sarcoidosis is a complex, multifactorial immune disorder with unknown etiology. A single nucleotide polymorphism (G→A, rs2076530) in the *butyrophilin-like 2* (*BTNL2*) gene results in a truncating protein formation. It has been previously reported that this variation may be a risk factor for sarcoidosis in certain ethnic groups. This study was conducted to determine whether there is any genetic predisposition for the *BTNL2* rs2076530 polymorphism in the 329-bp region in Turkish patients with sarcoidosis.

Materials and methods: DNA samples were obtained from volunteers including 53 Turkish patients with sarcoidosis and 52 healthy controls. Analysis of the 329-bp region was carried out by polymerase chain reaction and sequencing of genomic DNA.

Results: We did not find any genetic variation except the rs2076530 polymorphism in the 329-bp region. The AA genotype was associated with an increased risk of sarcoidosis in a recessive model [P = 0.027, OR 2.56 (95% CI 1.02–6.49)], but it did not include a risk for sarcoidosis in a dominant model (P = 0.885).

Conclusion: Our results emphasize the recessive characteristic of the rs2076530 polymorphism in Turkish patients with sarcoidosis. The lack of any genetic variation except rs2076530 in the 329-bp region is another significant finding for Turkish patients.

Key words: *BTNL2* gene, genetic predisposition, single nucleotide polymorphism, sarcoidosis

1. Introduction

Sarcoidosis is a multisystemic chronic inflammatory disorder that can affect the lungs, eyes, liver, and lymph nodes and is mainly characterized by accumulations of noncaseating epithelioid granulomas (1,2). The disease has a complex pathogenesis and the etiology still is unknown (2).

However, the disease is currently thought to be activated in genetically susceptible individuals as a consequence of exposure to various environmental and occupational, as well as infectious, agents. Following the first description of 2 German sisters with sarcoidosis, the studies examining the familial clustering of the disease in various populations have reported 2.7%–17% of index cases with another affected family member (3–5).

According to the estimations of A Case-Control Etiologic Study of Sarcoidosis, the familial relative risk adjusted for age, sex, relative class, and shared environment has been reported by Rossman and Kreider (6) and Buck and McKusick (7), who confirmed the significantly increased risk of disease among first- and second-degree relatives of sarcoidosis patients. They also showed the importance of racial differences in the familial aggregation of sarcoidosis cases (6–8).

Family-based studies have shown an association for both disease susceptibility and phenotype expression between sarcoidosis and human leukocyte antigen (HLA). Genome-wide linkage studies have pointed out that the extended major histocompatibility (MHC) locus on chromosome 6p was linked to susceptibility to sarcoidosis. However, because of the linkage disequilibrium in MHC, it is not clear whether the susceptibility to the disease is directly determined by HLA genes or if the association is resulting from other linked genes within this region. The truncating single nucleotide polymorphism rs2076530 in exon 5 of the *butyrophilin-like 2* (*BTNL2*) gene, a member of the immunoglobulin superfamily localized in close proximity of the HLA complex on chromosome 6p, has been implicated for the first time as a risk factor for sarcoidosis susceptibility by Valentonyte et al. (9). This

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G/A transition causes an alternative splice site located 4 bases upstream of the wild-type donor site. The premature truncation of protein disrupts the membrane localization of the protein because of the lack of the C-terminal of the IgC domain and transmembrane helix (9).

The *BTNL2* gene, an immunoglobulin gene superfamily member with 6 exons, is located in close proximity to the HLA class 2 and class 3 regions which is the most gene-dense, polymorphic region of the genome, containing predominantly immunomodulating genes (10). The protein encoded by the *BTNL2* gene shares considerable amino acid and domain structure homology with the B7.1 receptor. The B7.1 receptor, a member of the B7 costimulatory molecule family, plays an important role in the interaction between B and T lymphocytes (11). It can either inhibit or promote T cell responses, depending on the receptor it joins (12). Further studies in mouse models have shown that BTNL2 protein binds to a receptor on activated T cells and functions to suppress T cell proliferation (13). Therefore, the involvement of the truncated BTNL2 protein seems to be important in sarcoidosis immunopathogenesis.

The close proximity of the *BTNL2* gene to HLA-DRB1 and HLA-DQB1, which both have known sarcoidosis risk alleles, complicates association studies between *BTNL2* and sarcoidosis. Therefore, Rybicki et al. studied increased haplotype diversity in the MHC class II region in populations of African origin to differentiate specific gene effects (8). They identified 10 single nucleotide polymorphisms (SNPs) within a 490-bp region spanning exon/intron 5 of the *BTNL2* gene. They concluded that although haplotype variation within this region was significantly associated with sarcoidosis in all studied populations, the significance was higher in the white population. Subsequent studies reported conflicting results in different populations (14).

Our aim was to verify the association between the rs2076530 SNP and sarcoidosis in Turkish patients and to identify population-specific SNPs associated with sarcoidosis within the exon 5/intron 5 border.

2. Materials and methods

2.1. Subjects

The total subject group comprised 53 patients with sarcoidosis and 52 healthy Turkish individuals recruited from the Department of Chest Diseases of the Eskişehir Osmangazi University Faculty of Medicine in Eskişehir, Turkey. All patients fulfilled the criteria defined in the ATS/ERS/WASOG Statement on Sarcoidosis (1). Informed consent was obtained from all patients and healthy controls in accordance with our protocol, which was accepted by the Ethics Committee of the Eskişehir Osmangazi University Faculty of Medicine. In terms of ethnicity, the patients and the healthy controls were all homogeneously Turkish.

2.2. Genotyping

Genomic DNA was isolated from the peripheral leukocytes of the individuals using standard methods. The primers used in the amplification of the 329-bp exon 5/intron 5 region, including the rs2076530 polymorphism, were F- 5'-CTGGAGAGCAGATGGCAGAGTAC-3' and R- 5'-ATGACAGTTTCACACACTGGAGG-3'. The polymerase chain reaction (PCR) was carried in a total reaction volume of 50 µL with 25 µL of master mix and 10 ng/µL genomic DNA. The amplification protocol included initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 1 min, and final extension for 10 min. Quality control of PCR products was performed by agarose-gel electrophoresis to confirm the amplification sizes. Then, following the purification protocols, the PCR products of the *BTNL2* exon 5-intron 5 region including the rs2076530 polymorphism were sequenced by DNA cycle sequencing (Applied Biosystems 3130) using BigDye Terminator chemistry according to the manufacturer's protocols. The genotype was determined by Sequence Analysis 5.1 software. The genotype of each case was compared in the BLAST database for identification of sequence variation.

2.3. Statistical analysis

In the statistical analysis of allelic and genotypic distributions, SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA) was used. *BTNL2* variation frequencies in the groups were compared using Pearson's chi-square test. Odds ratios (ORs) were calculated to estimate the relative risk of sarcoidosis using logistic regression analysis. The 2-proportions test was used to analyze the frequencies of *BTNL2* genotype as a binary comparison.

3. Results

The sequenced 329-bp region of exon 5/intron 5 in each patient and control was evaluated in the BLAST database. The only detected variation in the sarcoidosis patients was the rs2076530 polymorphism; no further difference in the 329-bp region was detected. A statistically significant difference (P = 0.034) was seen when the frequencies of the GG, GA, and AA genotypes in the sarcoidosis group (32.1%, 24.5%, and 43.4%, respectively) were compared with the genotypes of the control group (30.7%, 46.2%, and 23.1%, respectively). The frequency of the AA genotype was significantly increased in the sarcoidosis group (Table 1).

No statistically significant difference (P = 0.168) was determined when the allele frequencies of sarcoidosis patients (G = 44.3%, A = 55.7%,) were compared with the controls (G = 53.8%, A= 46.2%) for the rs2076530

Table 1. Genotype and allele frequencies of the BTNL2 rs2076530 polymorphism of sarcoidosis patients and healthy controls.

Groups	N	Genotypes						Alleles			
		GG		GA		AA		G		A	
		N	%	N	%	N	%	N	%	N	%
Controls	52	16	30.7	24	46.2	12	23.1	56	53.8	48	46.2
Sarcoidosis patients	53	17	32.1	13	24.5	23	43.4	47	44.3	59	55.7
P-value		P = 0.034[a], P = 0.166[b], P = 0.234[c], P = 0.009[d]						P = 0.168[a]			

[a]Pearson's chi-square test for all groups, [b]GG vs. GA; [c]GG vs. AA; [d]GA vs. AA by using 2-proportions test.

polymorphism (Table 1). We found a statistically significant difference between the frequencies of the *BTNL2* genotypes of the patients and the controls, and so we analyzed these genotypes as a binary comparison. The frequency of the GG genotype was not statistically important compared with GA (P = 0.162) and AA (P = 0.230) genotypes. However, a significant difference was found between the frequencies of the GA and AA genotypes (P = 0.006).

According to the *BTNL2* genotype distribution, the risk of having sarcoidosis was calculated by the OR, and results are shown Table 2. The AA genotype was associated with an increased risk of sarcoidosis in a recessive model [P = 0.027, OR 2.56 (95% confidence interval [CI]: 1.02–6.49)] but was not associated with a risk in a dominant model (P = 0.885).

4. Discussion

In the present study, we aimed to sequence the 329-bp region of the *BTNL2* gene including the rs2076530 SNP in Turkish patients with sarcoidosis. In order to design our study, the literature was retrospectively searched. The first gene region to show genetic predisposition to sarcoidosis was identified by Valentonyte et al., who searched a 16.4-Mb locus on 16p21 and found a 15-kb section of *BTNL2* associated with sarcoidosis in German patients (9). Nguyen et al. published the first results of the *BTNL2* gene function, which is involved in T-cell activation on the basis of its homology with B7-1 (13). They reported the highest expression of *BTNL2* in mouse lymphoid tissues, as well as intestines, and enhanced the expression of its putative receptors on B and T cells. These expression analyses showed that the *BTNL2* molecule functions to inhibit T cell activation, which has impacts in immune diseases such as sarcoidosis. It is becoming more apparent that the gene function of *BTNL2* may play an important role in the pathophysiology of sarcoidosis. The accumulation of familial cases has also suggested that a genetic predisposition to the disease exists (3–5).

In this study, we evaluated the SNP of the *BTNL2* gene as a possible genetic risk factor for sarcoidosis in a Turkish population. In the complete patient cohort, *BTNL2* rs2076530 was significantly associated with disease risk. Many studies have been conducted in different ethnic groups in terms of genetic predisposition, but different clinical manifestations have been obtained. In white and African American populations, Rybicki et al. carried out family-based and case-control studies (8). They reported the strongest association of rs2076530 SNP with sarcoidosis. They also emphasized that the higher association between the rs2076530A allele and sarcoidosis was predominantly seen in the white population.

In another study, sarcoidosis patients and control subjects were recruited from 2 European countries (14). The A allele of *BTNL2* was associated with an increased risk

Table 2. Association of BTNL2 genotype with sarcoidosis susceptibility according to mode of inheritance.

Groups	N	Dominant model				Recessive model			
		AA/GA		GG		AA		GA/GG	
		N	%	N	%	N	%	N	%
Controls	52	36	69.23	16	30.77	12	23.08	40	76.92
Sarcoidosis patients	53	36	67.93	17	32.07	23	43.40	30	56.60
P-value		P = 0.885*				P = 0.027*			

*Pearson's chi-square test.

of sarcoidosis in these populations when the patient group was evaluated as a whole. However, it was not associated with sarcoidosis when the exclusion criterion was present, which clinically and genetically represented a distinct disease subset (14,15). Li et al. carried out a case-control study including German patients, similar to the study by Valentonyte et al. (9,16). According to their results, the A allele is associated with an about 2-fold higher risk of sarcoidosis in both a codominant and a dominant model, but not in a recessive models. Another important study performed by Milman et al. was added to the studies of different ethnic groups (17). Their study comprising Danish patients was significant in terms of ethnic data and different levels of risk for sarcoidosis. The *BTNL2* A allele variant was very frequent in Danish patients with sarcoidosis, and the AA genotype was associated with a 3.1-fold higher risk of sarcoidosis than the GG genotype. Morais et al. evaluated the *BTNL2* rs2076530 G/A allele associations with sarcoidosis susceptibility in a Portuguese population (18). Their data replicated the association of *BTNL2* rs2076530 A allele with sarcoidosis. In contrast to Rybicki et al., a genome-wide association study conducted in African-Americans showed that the A allele was associated with higher risk of sarcoidosis (8,19). Suzuki et al. performed a study in order to assess whether the *BTNL2* association was independently present in Japanese patients with sarcoidosis (20). The *BTNL2* rs2076530 A allele was associated with an increased risk of sarcoidosis (OR = 1.84) in their study, but the OR was lower than in other ethnic groups.

In accordance with these results, our hypothesis was that there might be a new variation around this mutation. The 329-bp region was sequenced, but no variation was found except the rs2076530 polymorphism in Turkish patients with sarcoidosis. According to our results, there was a significant association between the *BTNL2* AA genotype and sarcoidosis in Turkish patients. However, we did not find a statistically significant difference when the A allele frequency in the sarcoidosis group was compared with the controls. In terms of the A allele frequency, we observed a visible difference between sarcoidosis patients and control subjects, but it did not indicate statistical significance.

In conclusion, we found that the AA genotype was associated with a 2.5-fold higher risk of sarcoidosis than other genotypes in a recessive model. However, there was no statistical difference in a dominant model. Milman et al. reported that the AA genotype is associated with an increased risk of sarcoidosis in both a dominant and recessive model (17). Because of the sample size of our study and difference in studied ethnic groups, our results may have differed from theirs. The present study has certain limitations in order to determine hereditary characteristics but is important in terms of being the first report in a Turkish population. Our results emphasize the recessive characteristic of the rs2076530 polymorphism in Turkish patients with sarcoidosis. The lack of any genetic variation except rs2076530 in the 329-bp region is another significant finding for Turkish patients.

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