

Detection of *BTNL2* Gene Mutation (rs2076530 Allele) in Iranian Sarcoidosis Patients: A clinical and Genetic Study

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Received date: October 24, 2016; Accepted date: May 05, 2017; Published date: May 15, 2017

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Abstract

Background/Aims: Sarcoidosis is a multiorgan granulomatous inflammatory disease of an unknown cause, probably due to inappropriate T-cell response. Mutation in *BTNL2* gene (Butirophylin-like2) which is one of the most important genes in MHC II (complex tissue incompatibility) group is related to sarcoidosis. Our purpose was to evaluate *BTNL2* rs2076530 G/A allele as a putative genetic risk for sarcoidosis in an Iranian population.

Methods: DNA from patients and controls was obtained from peripheral blood using standard methods. 490-bp amplicon of each samples were genotyped for the *BTNL2* G → A transition of rs2076530 using an ABI 3130 automated sequencer-Big-Dye Terminator Version 3.1 Cycle.

Results: A total of 50 patients with sarcoidosis were registered in our study of which 33 were females (66%) and 17 were male (34%). 26 women (52% total) and 14 men (28% of total) showed SNP mutation in Exon/Intron 5 of the *BTNL2* gene (P Value<0.001). In these groups of patients, 40 (80%) had G to A transition at rs2076530 allele and 10 patients (20%) didn't have the mutation. 40 control samples were checked as control and all of them were normal for this allele.

Conclusion: Our findings in clinical and also genetically, indicates that rs2076530 allele is a "high risk" criterion in Iranian sarcoidosis.

Keywords: Iranian sarcoidosis patients; *BTNL2* gene; rs2076530 allele; Molecular diagnostics

Introduction

Sarcoidosis, is a multi-systemic disorder of unknown cause characterized by the formation of immune granulomas in affected organs, probably results from an exaggerated T-cell response to an air borne antigen (American Thoracic Society et al.) [1]. Whereas human leukocyte antigen (*HLA*) genes have long been thought to play a role in sarcoidosis (Martinetti et al.) [2], The high density of immune major histocompatibility complex (MHC) region create difficulties in separating out individual gene effects. Following-up a previously detected *HLA* linkage to sarcoidosis (Schurmann et al.) [3], (Valentonyte et al.) [4] reported a novel association with rs2076530 allele, a coding SNP on exons of the *BTNL2* gene (MIM606000) that is independent of *HLA-DRB1* sarcoidosis risk alleles.

This gene is located on chromosome 6P21.3 which has 6 exons. The rs2076530 G to A transition leads to an alternative splice site those results in an early stop codon and a truncated protein. *BTNL2*, aliases Butyrophylin like 2 and *BTL-2* is a butyrophylin gene that belongs to

the immunoglobulin gene superfamily and is related to the B7, 1 and B7, 2 (CD80 and CD 86) costimulatory receptors (Rhodes et al. [5], Sharpband et al.) [6], but its exact function is unknown. Optimal T-cell activation requires antigen engagement of the T-cell receptor with additional costimulatory interactions. To determine the consistency of the rs2076530 allele in *BTNL2* gene as sarcoidosis risk factor in Iranian sarcoidosis patients we characterized the mutation in the Exon/Intron 5 region of *BTNL2* in Iranian patients' samples that consisted of 50 sarcoidosis patients and 40 normal samples as a case control.

Clinical Presentation

The presentation of sarcoidosis depends on epidemiological factors such as age, sex and race, the duration of the disease (Baughman et al.) [7]. The incidence is globally estimated at around 16.5/100,000 in men and 19/100,000 in women (Rybicki et al.) [8]; the life time incidence is higher in women (1.3%) than in men (1%) and in blacks (2.4%) than in Caucasian (0.8%) (Sartwell et al.) [9].

Sarcoidosis is mostly revealed in the following circumstances: respiratory symptoms, firstly persistent dry cough in around 30% cases, extrathoracic localizations, mainly peripheral lymph nodes, eyes

or skin, constitutional symptoms such as fatigue (27%), weight loss (28%), fever (10% to 17%) or night sweats, erythema nodosum (3% to 44%) (Hunninghake et al.) [10], Intrathoracic is the most frequent involved organ in sarcoidosis, chest X-ray is abnormal at around 86% to 92% of cases and remains a key investigation for diagnosis.

Radiographic staging of sarcoidosis is based on the presence of lymphadenopathies and lung infiltration without or with fibrosis (Costabel et al.) [11]. Lymphadenopathies are typically hilar, bilateral, symmetrical and non-compressive, and often associated with right Para tracheal and aortic-pulmonic window lymph node involvement (Judson et al.) [12]. Stage 1, the most frequent (55% to 90%) presentation, is defined as isolated intrathoracic lymphadenopathy, Stage 2 (40% to 70%) as lymphadenopathy accompanied by lung infiltration and Stage 3 (10% to 20%) as lone parenchymal infiltration and stage 4 (0%) refers to overt lung fibrosis (Pietinalho et al.) [13].

Typical stage 1 and 2 are highly reliable for sarcoidosis diagnosis, while stage 3 and 4 are far less accurate. Peripheral lymphadenopathies are easily palpable and their frequency varies between series up to 70% they are asymptomatic (Reich et al.) [14], firm, of various size and every site of lymph nodes may be affected. The frequency of ocular sarcoidosis is between 10 and 50% according to published studies (Zych) [15]. Any part of the eye may be involved in sarcoidosis. Macroscopic nodules of the conjunctive are seen in 6% to 40% of cases and allow evidence of granulomatous in 67% if cases. Skin manifestations of sarcoidosis are heterogeneous (Mahajan et al.) [16].

The frequency of specific skin manifestations of sarcoidosis ranges from 10% to 40% cases (Ahmed et al.) [17]. And cardiac involvement of sarcoidosis s can occur at any time during the course of sarcoidosis (Duke et al.).

The left ventricular myocardium and more specifically, the interventricular septum and the free left lateral wall are the most frequently involved structures in sarcoidosis (Sekiguchi et al.) [18], any part of the nervous system can be involved in sarcoidosis with a frequency around 10%. Hypercalcemia is present in around 11% and serum angiotensin converting enzyme (SACE) is believed to reflect disease activity but it is increased in about 60% of cases (McLean et al.) [19]. To demonstrate manifestations of sarcoidosis symptoms in Iranian patients, we checked all clinical features including; Lung problems, Dermatological study, Cardiopathy, Ophthalmopathy, Neuropathy, measurement of ACE enzyme and theirs frequencies for all patients as show in Figure 1.

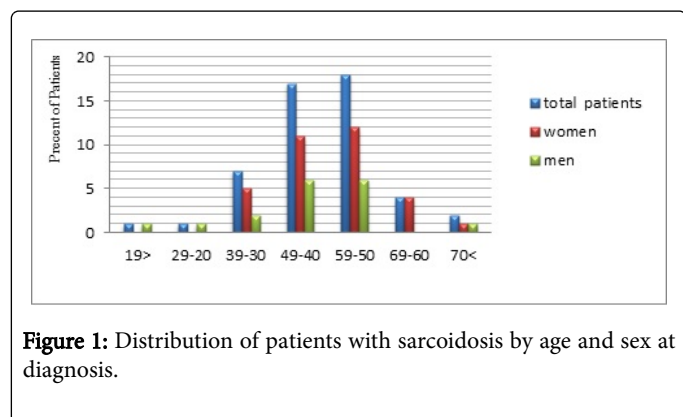


Figure 1: Distribution of patients with sarcoidosis by age and sex at diagnosis.

Mutation Analysis

Total genomic DNA (50 sarcoidosis patients were registered in our study together with 40 control) were obtained (with informed consent) from peripheral blood leukocyte. Custom primer oligonucleotide for PCR and DNA directly sequencing were designed from the rs2076530 sequence, which including;

Forward: 5AATGCACAGAGCATGGAGGTGAG-3 And

Reverse: 5-GAAGATACTGGAAAAGATACAAG-3.

To amplify a 490-bp amplicon from each of amplification and PCR amplification of genomic DNA was performed by standard PCR protocols. All PCR reactions were performed using 40 ng of genomic DNA, 200 μM of dNTPs and 0.5 μM of primers under the following conditions: initial denaturation for 5 min at 95°C followed by 35 cycles of three steps, 95°C for 30 sec, 62°C as a annealing temperature for 30 sec, 72°C for 45 second and a terminal extension for 10 min at 72°C. After a quality check of PCR products by electrophoresis on 1% agarose gel, Sequencing was completed on an ABI 3130 automated sequencer (XL Genetic Analyzer) using the Big-Dye Terminator Version 3.1 Cycle.

Results

A total of 50 patients with sarcoidosis were registered in our study, at the sex distribution (Figure 1), 33 (66%) patients were females and 17 (34%) patients were male. The most frequent involved age was between 50 years to 59 years (figure 1). In the clinical features distribution 43 (86%) patients had Lung problem including 31 (62%) patients stage 1, 8 (16%) stage 2, 3 (6%) stage 3, 3 (6%) bronchopathy and 3 (6%) pleuritis (Table 1).

Roentgen graphic stage	Number	Percentage	% G to A Transition
Stage 1	31	62	80.645
Stage 2	8	16	87.5
Stage 3	3	6	66.66
Bronchopathy	3	6	33.33
Pleurit	3	6	33.33

Table 1: Characteristics of lung involvement of patients with the percent of mutation distribution.

Dermal reactions were the other clinical feature, including 18 (36%) case of erythema nodosum, 3 (6%) lupus pernio and 20 (40%) case of different dermal patterns; arthralgia in 8 (16%) patients; 5 (10%) case with parotitis; 2 (4%) with sinusitis; 4 (8%) with peripheral lymphadenitis; 3 (6%) with thrombocytopenia; 4 (8%) with cardiopathy; 5 (10%) with ophthalmopathy and 4 (8%) with neuropathy and 32 patients (64%) had an increased ACE (Table 2).

Clinical Findings	Number	Percent
Pneumopathy	43	86
Dermopathy	20	40
Erythema nodosum	18	36
Arthralgia	8	16

Parotitis	5	10
Sinusitis	2	4
Peripheradenitis	4	8
Lupus Pernio	3	6
Thrombocytopenia	3	6
Cardiopathy	4	8
Ophthalmopathy	5	10
Neuropathy	4	8
Increased ACE	32	64

Table 2: Number and percentage of patients with specified clinical findings.

In the gene analysis study, 26 women (52% of total patients) and 14 men (28% of total patients) showed SNP mutation in Exon/Intron 5 of the *BTNL2* gene. In these groups of patients, 40 patients (80%) had G to A transition at rs2076530 allele and 10 patients (20%) didn't have the mutation. The most frequent ages that showed mutation were between 40 years to 49 years (Figure 2).

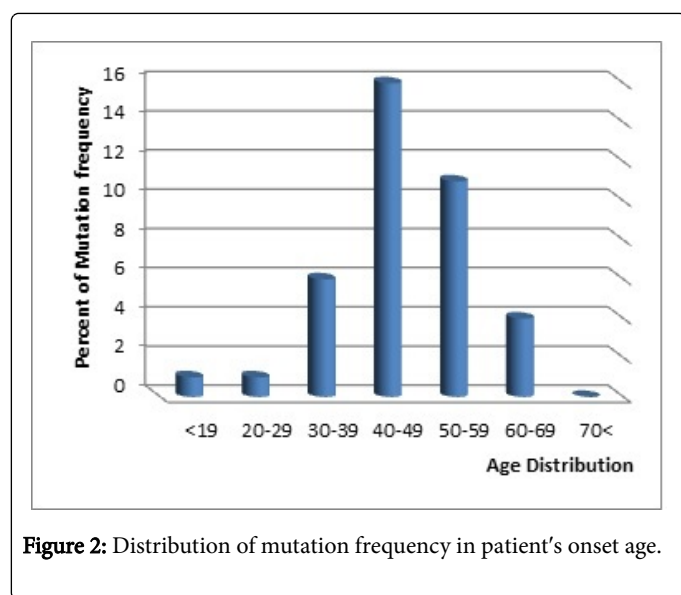


Figure 2: Distribution of mutation frequency in patient's onset age.

Also, we checked 40 control samples with the same characters in sex, (20 male and 20 female) for this allele, that subsequently they did not show any mutations in this allele.

Discussion

To the best of our knowledge, this is the first genetic analysis of *BTNL2* gene on the patients with typical clinical characterizations of sarcoidosis in Iran. In our study we had 50 Iranian typical sarcoidosis patients. Although, sarcoidosis can occur at all ages but a predilection is observed between 25 years to 40 years in both genders at least in Scandinavian countries and Japan (Morimoto et al.) [20] and a second peak of incidence has been reported in women over 50 years of age in some but not all published series, whereas in our study, the ages were 49-59 and women were more affected. 48 patients (96%) had intra

thoracic problems including 62% at stage 1, 16% at stage 2, 6% at stage 3, 6% with bronchopathy, and 6% with pleuritis. The most extrathoracic findings were dermatological reactions in 41 of 50 (82%) patients including 18 (36%) case of Erythema Nodosum, 3 (6%) case of Lupus Pernio and 20 (40%) patients had different dermal findings. Other extrathoracic affected organs are mentioned above. 80% of patients (P value<0.001) had a G to A transition in rs2076530. In a recently published genetic study, sarcoidosis clinical presentation has been linked to a truncating splice variant in the *BTNL2* gene (Rhodes et al.) [5]. The authors reported that a point mutation in the *BTNL2* gene introduces a cryptic splice site located 4 base pairs upstream of the affected wild-type donor site that generated a mutant protein with a premature stop codon. The truncated *BTNL2* protein lacks a membrane anchoring domain and exhibits disrupted membrane localization (Coudurier et al.) [21]. *BTNL2* is expressed in cells of the immune system and has been implicated as a receptor molecule involved in the control of T-cell proliferation. Loss of membrane localization appears to impair the inhibitory immunoregulatory function of *BTNL2*. Thus the altered intracellular distribution of mutant *BTNL2* may account for the exaggerated cellular immune response and increased inflammatory activity of macrophages seen in sarcoidosis (Coudurier et al.) [21]. In conclusion, sarcoidosis is a multifaceted disease. our results show that organ involvement differed according to sex and age and the mutation mentioned above is a favorite index of sarcoidosis in Iranian patients; Our findings indicates that rs2076530 allele in *BTNL2* gene is a "high risk" criterion and is in accordance clinically and genetically with the results of some other studies around the world.

Acknowledgment

The authors wish to thank all patients and physicians for their cooperation. Also the authors kindly thank Dr Mostafa Bigdeli (Head of Masood laboratory) and Dr Pirooz Salehian (Head of the Modern pathology Department), for expert technical help.

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