

# Polymorphism of rs2076530 allele in BTNL2 gene in patients with skin sarcoidosis compared to skin sarcoidal reaction and normal skin: A case-control study

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Conflicts of interest: None to declare

Received: 30 June 2016  
 Accepted: 27 July 2016

## INTRODUCTION

Sarcoidosis is a granulomatous disorder with variant clinical manifestations accompanied by the involvement of different organs such as the kidney,

**Background:** In 10-30% of the cases with sarcoidosis, skin lesions appear solely without any systemic signs or symptoms. BTNL2 gene, which is a member of the immunoglobulin gene super family and is associated with CD86 and CD80 co-stimulatory receptors, is identified to play an important role in the establishment of sarcoidosis. We aimed to evaluate the role of this gene in patients with skin sarcoidosis in comparison with skin sarcoidal reaction patients and those with normal skin.

**Methods:** The nucleotide sequence of rs2076530 allele in exon 5 of BTNL2 gene was compared among the paraffin-embedded blocks of 34 patients with a histologic diagnosis of sarcoidosis, 14 patients with skin sarcoidal reaction (tattoo, foreign body), and 27 patients with normal skin (excised during cosmetic surgery) using polymerase chain reaction.

**Results:** There was no statistically significant difference in the frequency of 3 genotypes of AA, AG, and GG in rs2076530 allele among skin sarcoidosis, skin sarcoidal reaction, and normal skin.

**Conclusion:** The expression of rs2076530 allele of BTNL2 gene in skin sarcoidosis or sarcoidal reaction does not differ with its expression in the normal skin.

**Keywords:** BTNL2 gene, mutation, cutaneous sarcoidosis.

Iran J Dermatol 2016; 19: 86-88

liver, lung, and heart. The early symptoms of this disease often occur as pulmonary involvement together with signs of the involvement of the lymphatic system. Different studies have evaluated the etiologic factors of sarcoidosis; however, the

etiology remains unknown<sup>1,2</sup>. Skin lesions appear solely before any systemic signs or symptoms in 10-30% of the cases with sarcoidosis<sup>3</sup>. Evidence of ethnic predisposition to sarcoidosis and higher prevalence in some families support the concept that genetic factors contribute to the development of the disease<sup>4-6</sup>. BTNL2 gene, which is a member of the immunoglobulin gene super family and is associated with CD86 and CD80 costimulatory receptors, is identified to play an important part in the establishment of sarcoidosis<sup>7</sup>.

In a preliminary study, A to G transition was observed in 7 out of 10 skin sarcoidosis samples<sup>8</sup>. Considering the association between BTNL2 gene and sarcoidosis, and with regards to the importance of precise diagnosis of the disease<sup>9</sup>, we conducted this study to evaluate the role of this gene in patients with skin sarcoidosis.

## PARTICIPANTS AND METHODS

Skin tissue samples of 34 white Iranian patients with clinical, histologic, and laboratory diagnosis of sarcoidosis and 14 white Iranian patients with sarcoidal skin reaction (tattoo, foreign body) were evaluated and the nucleotide sequences of the rs2076530 allele in exon 5 of BTNL2 gene were compared with those of 27 normal skin tissue samples (removed during esthetic surgery) using polymerase chain reaction according to the method previously described<sup>8</sup>.

The following primers were used:

BTNL2 Forward:

5'CAGTTTGGATCTGAAGGTGCTA3'

BTNL2 Reverse:

5'TCATCCATTGAGTTTGTGGA3'.

The results were analyzed by SPSS 16 (SPSS Inc., Chicago, IL, USA). Genotypes of AA, AG, and GG were compared among three groups (skin sarcoidosis, sarcoidal reaction, and normal skin) using chi square test.

## RESULTS

The frequency of 3 genotypes of AA, AG, and GG in the rs2076530 allele in skin sarcoidosis, sarcoidal reaction, and normal skin is shown in Table 1. No significant difference was found in frequency of these alleles either between skin sarcoidosis and normal skin ( $P=0.86$ ), or skin sarcoidal reaction and

**Table 1.** The frequency of three genotypes of AA, AG, and GG, in rs2076530 allele in skin sarcoidosis, sarcoidal reaction, and normal skin

Diagnosis	AA	AG	GG	Total
Sarcoidosis	17	12	5	34
Sarcoidal reaction(Tattoo, foreign body)	5	6	3	14
Normal skin	14	8	5	27

**Table 2.** Pair-wise correlation evaluation between skin sarcoidosis, normal skin, and sarcoidal reaction.

	Correlation Coefficient ( <i>r</i> )	<i>P</i>
Sarcoidosis vs. normal skin	0.0688	0.865
Sarcoidal reaction vs. normal skin	0.1565	0.650
Sarcoidosis vs. sarcoidal reaction	0.1328	0.650

normal skin ( $P=0.59$ ). Furthermore, there was no significant difference between skin sarcoidosis and sarcoidal reaction in this regard ( $P=0.65$ ) (table 2).

## DISCUSSION

Early diagnosis and treatment of sarcoidosis, considering the probability of its development to a systemic form, is of great importance. The skin is involved in 50% of the cases; however, sarcoidosis may occur in the heart and central nervous system in 5-10% and in parotid glands in 4-6% of the patients<sup>8</sup>. Skin manifestations include firm and purplish plaques and erythema nodosum, which usually occur as non-specific lesions during the disease course. Clinical evaluations, laboratory analysis and pathologic tests have been routinely used for the diagnosis of sarcoidosis. However, difficulties in diagnosis have been demonstrated in cases with unusual manifestations or uncommon organ involvement<sup>1</sup>. A number of studies have reported that the A allele of rs2076530, a single nucleotide polymorphism in exon 5 of BTNL2 gene, is closely associated with a higher risk of sarcoidosis development. In addition, HLA function plays an important role in the development and progression of the disease. BTNL2 is one of the crucial genes in major histocompatibility complex (MHC) class II molecules<sup>10</sup>. This gene is related to B7.1 and B7.2 receptor families that are probably co-stimulatory molecules for T-cells<sup>11</sup>. BTNL2 gene is located on chromosome 6p21.3 and includes 6 exons. The rs2076530 allele is located on exon 5 of BTNL2; Rybicki *et al.* reported this allele as a risk factor

for sarcoidosis. A transition type mutation, which alters G to A, results in the production of this allele and leads to an early stop codon and a truncated protein with an abnormal function<sup>12</sup>. Recent studies have demonstrated that BTNL2 gene is produced in the intestine and lymphatic tissues and plays an important role in the activation of T-cells and therefore in immunologic diseases, sarcoidosis, and myositis<sup>13</sup>. Studies on sarcoidosis in Britain and the Netherlands have analyzed rs3763309 (C/A Intron 1), rs2076523 (A/G Intron 3), rs2076524 (T/C Intron 4), and rs3117099 (T/C 3' untranslated region) and concluded that G allele (rs2294878) (65.6/55.3) and A allele (rs2076530) (66.8/57.04) were more frequent in patients with sarcoidosis. Other alleles were not significantly related to sarcoidosis. In addition, HLA DRB1\*14, HLA DRB1\*01, HLA DRB1\*12, HLA DRB1\*10, and HLA DRB1\*04 alleles had a significant relation with sarcoidosis<sup>14</sup>. In a case-control study, the role of BTNL2 gene in sarcoidosis was evaluated in patients of African American origins. The results confirmed an association between BTNL2 and sarcoidosis in white patients. In addition, it was found that the role of BTNL2 gene in sarcoidosis was independent of HLA class II in whites but could interact antagonistically in African Americans<sup>12</sup>. We found no significant interaction between genotype and histopathologic outcomes in skin samples of either normal or sarcoidosis patients. Therefore, additional studies are suggested in order to find the association of rs2076530 allele with skin sarcoidosis and its role in determination of the prognosis of systemic sarcoidosis.

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