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Homozygous variant rs2076530 of BTNL2 and familial sarcoidosis

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Abstract. *Rationale:* Despite extensive studies, the pathogenesis of sarcoidosis is largely unknown. Although multiple environmental and putative infectious agents have been proposed, none was retained as a major contributor to the disease occurrence. Genetic predisposition to sarcoidosis was considered as a significant factor and numerous candidate genes have been reviewed. This last point was reinforced since the discovery of a pathogenic polymorphism (rs2076530 or G >A) of the *BTNL2* gene, leading to an early truncation of the protein, which increases the relative risk of the disease. *BTNL2* is known to act as a co-stimulatory molecule, inducing a negative signal to T-lymphocyte activation and the mutated gene is responsible for a truncated protein and disruption of membrane localization. *Objectives:* Our work attempted to confirm this observation in a highly penetrant familial form of sarcoidosis. *Results:* In this family, the disease was diagnosed in 5 members through 3 generations. Despite individual clinical specificities, all displayed severe forms of the disease. Peripheral blood samples were collected from 3 patients and 2 additional healthy children of the fourth generation. Analysis of the *BTNL2* gene confirmed the presence of the pathogenic variant of BTNL2 on both alleles (A/A homozygous genotype) in all subjects tested. *Conclusions:* Our data suggest that the absence of a membrane anchored BTNL2 protein may increase genetic susceptibility to sarcoidosis and familial occurrence of the disease. This observation assessed the putative pathogenic involvement of the rs2076530 variant of *BTNL2* in the development of this granulomatosis disease. *(Sarcoidosis Vasc Diffuse Lung Dis 2009; 26: 162-166)*

Key words: familial sarcoidosis, BTNL2, rs2076530 SNP, co-stimulatory molecule

Introduction

The pathogenesis of sarcoidosis remains poorly understood. However, the uncommon occurrence of familial forms of the disease suggests a putative genetic susceptibility to develop sarcoidosis, even if the disease is likely multifactorial (1). According to a recent study, a single nucleotide polymorphism (SNP) in the butyrophilin-like 2 (*BTNL2*) gene (rs2076530) was closely associated with sarcoidosis (2). Among numerous candidate genes, which may confer disease susceptibility, *BTNL2* might be of a particular interest. Sequence homology studies revealed that *BTNL2* belongs to the B7 family of co-stimulatory molecules and likely functions to down-regulate T-cell activation (3, 4). To assess the role of the *BTNL2* gene in familial sarcoidosis, we searched the BTNL2 related rs2076530 polymorphism in a family showing a highly penetrant and severe disease, involving 5 patients.

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In this family, we identified a homozygous state of the putative pathogenic SNP, referenced as G to A variant at donor splice site of exon 5 and intron 5. This observation suggests that the full absence of a normal membrane anchored BTNL2 protein might be a significant factor in genetic susceptibility to sarcoidosis.

Case reports

The family pedigree tested in this report was described in Figure 1. In 1966, a 26 year old Caribbean native woman (II.3), was seen for recurrent ocular symptoms, consistent with an uveitis. Respiratory symptoms were absent but a concomitant chest X-ray demonstrated a diffuse pulmonary infiltration and enlargement of mediastinal lymph nodes. The diagnosis of sarcoidosis was highly suspected. Corticosteroid treatment was rapidly introduced and led to an excellent initial response. However, during the following decades, the patient developed a progressive respiratory and kidney failure, related to severe and progressive lung fibrosis and chronic hypercalcemia, respectively. The follow-up was marked by the occurrence of a splenomegaly requiring a splenectomy, allowing a pathological diagnosis of sarcoidosis. The updated follow-up of II.3 shows that using a continuous corticosteroid therapy, sarcoidosis is stable, without significant decline of lung and kidney functions.

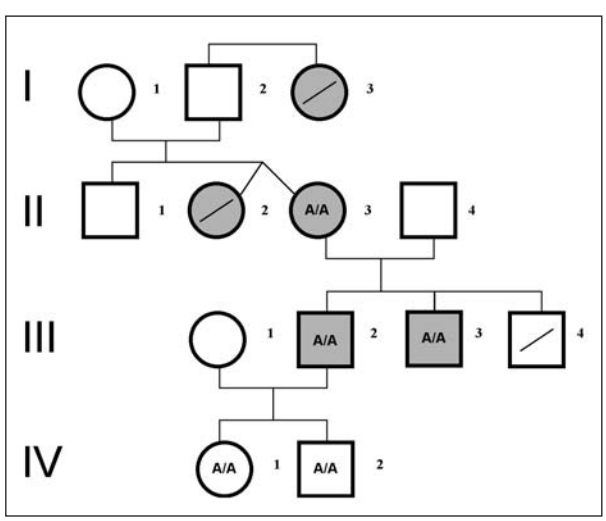

Fig. 1. Genealogical tree of the familial sarcoidosis. Circle: female, square: male, closed symbols: subject with sarcoidosis, open symbols: subjects without sarcoidosis, dashed symbols: dead patients

Simultaneously in 1966, her twin sister (II.2) was hospitalized for a multi-systemic sarcoidosis, with stage III pulmonary lesions, involving the liver and the spleen. Despite corticosteroids, outcomes were rapidly unfavorable. She died 16 years later, the cause of death being related to chronic respiratory failure.

In 1987, their paternal aunt (I.3), a seventy-eight year old woman presented with dyspnea. Chest X-ray displayed a retractile lung fibrosis, suggestive in this familial context, of a stage IV pulmonary sarcoidosis. Gallium scintography showed an intense fixation of mediastinal lymph nodes and spleen. Despite the employment of corticosteroids, a fatal evolution occurred within a few years, without definitive pathological confirmation of the diagnosis.

In 1996, a 32 year old man (III.2), the son of the index patient (II.3), complained of asthenia and dry cough. Clinically, he presented subcutaneous nodes and the chest X-ray showed a stage II pulmonary injury, with enlarged mediastinal lymph nodes. Pathological examination of the mediastinal lymph nodes was concordant with the diagnosis of sarcoidosis. Corticosteroid therapy induced clinical and radiological improvement. Initial outcomes were complicated by several relapses, when corticosteroids were tapered. Recent follow-up showed that despite several years without corticosteroids, the disease was not progressive. His two children (IV.1 and IV.2), six and eight years old respectively, remained asymptomatic to date.

The brother of III.2, a thirty-eight year old man, referenced III.3, was admitted to an emergency care unit in 2004 for acute abdominal and lumbar pain, further shown as a consequence of enlargement of multiple abdominal and retroperitoneal lymph nodes. Sarcoidosis was pathologically proved on an inguinal lymph node biopsy. Concomitant stage II pulmonary disease was present. Corticosteroid therapy was rapidly successful, then progressively decreased in 18 months. The updated follow-up identified no relapse.

Finally, the last brother, III.4, related to patients III.2 and III.3, had a striking history of sudden death at the age of 28 years. No information or diagnosis were available for him.

Genetic studies of BTNL2

Peripheral blood samples were collected from 5 members of the family (II.3, III.2, III.3, IV.1 and IV.2) available for this study. DNA was extracted from peripheral blood leukocytes by QIAamp DNA Blood mini kit (Qiagen). The *BTNL2* exon 5-intron 5 region surrounding the rs2076530 polymorphism was sequenced using Applied Biosystems BigDye chemistry and a 3130*xl* genetic analyzer in accordance with the supplier's recommendations. The following primers were used for PCR amplification and sequencing: BTNL2_5cF forward 5' to 3': CAGATGGCAGAGTACAGAGG and BTNL2_5cR reverse 3' to 5': AAGGACCTGTTAAAGAGACT. Amplification cycles consisted of an initial denaturation at 94°C for 5 min, then 30 cycles at 94°C for 30 s, 56°C for 30 s, then followed by 72°C for 1 min. For all the patients and relatives tested in the family (II3, III2, III3, IV1, IV2), a constant A/A homozygosity was found for the rs2076530 SNP variant of *BTNL2*. The 4 bp *GTAA* frameshift inducing deletion induced by the rs2076530 (G > A) variant was confirmed by sequencing the cDNA produced by RT-PCR of total RNA extracted from peripheral blood lymphocytes of patients and control individuals, using a set of forward 5' > 3' GTGTATATGGATGGGGGACCA and reverse 3' > 5' CTGGGAAGATGATGGTATCG primers producing a 345 bp product size and according to previously published protocols (2).

Discussion

Since the initial report of sarcoidosis in siblings (5), the description of additional several hundred relatives with the disease has pointed out the role of a genetic background in the development of sarcoidosis (1, 6-9). Occurrence of familial sarcoidosis is currently considered as the strongest support for a genetic component of the disorder (10).

The ACCESS study, performed on a large series of 10,862 first degree and 17,047 second degree relatives organized around 706 sarcoidosis case-control pairs, estimated that a first degree relative to an index case may have an odds ratio of 3.8 to develop the disease, when compared to control population (11). Nevertheless, familial occurrence of sarcoidosis is usually reported for only 2 first degree related members in a single family and rarely for more than 3 members. A retrospective study of 43 families (93 observations) reported 36 families with 2 members affected, and only 7 families showing 3 cases of sarcoidosis (12). The most frequent associations were in siblings (24 instances) and mother-children (14 instances) (12). Elford *et al* reported 5 cases of familial sarcoidosis with 3 linked cases between mothers and children and one atypical husband-wife association (13). Additionally, it has been shown that population subgroups had particular characteristics. Siblings and parents of Afro-American sarcoidosis probands had a 2.5-fold increased risk for sarcoidosis compared with the control population in the same ethnic subgroup (14). Our observation of 5 cases of severe sarcoidosis occurring in 3 generations of a single family is one of the largest familial aggregations described to date and may suggest an autosomic dominant mendelian inheritance.

In familial sarcoidosis, it has been proposed that clinical presentation, outcome of the disease and response to treatment shared similarities in siblings (15, 16). A recent study, done on 509 Afro-American siblings, tested the hypothesis that sibling pairs might have similar phenotypic disease. It has been solely demonstrated that the relative risk of a specific lesion, such as ocular or liver involvement was 3 times higher in first degree relatives of the index case. However, the study failed to show any other concordances (17). Although our family cases presented different clinical and radiological phenotypes, a trend towards severe sarcoidosis, characterized by chronic evolution, dependence on corticosteroids, and frequent occurrence of respiratory failure with fatal outcomes was noted. Interestingly, an association between chronic forms of the disease and A allele carriers of rs2076530 has been previously demonstrated (18).

Despite extensive research and multiple hypotheses, causative factors of sarcoidosis remain obscure (19-25). However, both observation of familial sarcoidosis and ethnic differences in incidence of the disease add weight to the potential responsibility of an inherited predisposition for its development (10, 26, 27). In the search for genes that may confer susceptibility to sarcoidosis, the genome of human leukocyte antigens and the sequence of lymphocyte activation might be of particular interest. Indeed, the

pathogenesis of sarcoidosis is partly characterized by an exaggerated cellular immune response, with an oligoclonal lymphocyte expansion, leading to granuloma formation (28). The butyrophilin-like 2 molecule (BTNL2), encoded by *BTNL2* on chromosome 6p21 shares sequence homology with the B7 molecule, and is thought to be engaged as a lymphocyte co-stimulatory molecule (2). Firstly, it has been shown that BTNL2 was able to inhibit mouse T-cell proliferation and TCR activation (4). Secondly, BTNL2 was described as a down-regulator of T-cell activation *in vitro*, acting as a negative co-stimulatory molecule and thus involved in inflammatory disease (29). Valentonyte *et al* showed that both familial and sporadic sarcoidosis were associated with a single nucleotide polymorphism in the butyrophilin-like 2 (BTNL2) gene, so referenced rs2076530. This SNP (G/A) was found at the 3' boundary of the exon 5 coding region. The A allele causes the usage of an alternative donor site at the exon 5–3' intron boundary leading to a 4 bp deletion at the mRNA level, frameshift, and premature truncation at the protein level. The resulting protein lacks the C-terminal IgC domain and trans-membrane helix, thereby disrupting the membrane localization of the protein (2). In this way, the homozygous mutation of BTNL2 (rs2076530; G > A) might induce a dysfunction of the negative co-stimulatory molecule BTNL2, responsible for a misregulation of T-cell activation in sarcoidosis. Although this later hypothesis remains to be proved, our data showing exclusively rs2076530 variant of BTNL2 in tested subjects underlines again its putative role in sarcoidosis pathogenesis.

In summary, a highly demonstrative family of sarcoidosis is reported, affecting 5 members in 3 generations. The youngest children of the last generation were healthy and shared the same *BTNL2* A/A genotype as expected. In that way, they may be considered as highly predisposed to sarcoidosis and will need careful follow-up in the future. The fact that rs2076530, G to A SNP in *BTNL2* is not considered to date as a high risk related mutation, but only as a low or moderate risk inducing SNP, is not discordant with the fact that being homozygous for the deleterious variant may confer an increased risk of granulomatosis development. Further studies, as those performed in France through a National Clinical Network (grant from PHRC–D50604) will assess the role of *BTNL2* by comparing a significant series of highly penetrant and/or severe familial forms to sporadic cases of sarcoidosis and healthy control population. Finally, defining the A/A genotype of BTNL2 related rs2076530 SNP, as a putative prognosis and/or predictive factor of recurrent and severe sarcoidosis might be helpful in the clinical management of the patients.

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