



Brief report

***NOD2* genetic variants and sarcoidosis-associated uveitis[☆]**

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ABSTRACT

Purpose: Identifying genetic risk factors for developing sarcoidosis-associated uveitis could provide insights into its pathogenesis which is poorly understood.

We determine if variants in *NOD2* confer an increased risk of developing uveitis in adults with sarcoidosis.

Methods: In this genetic case-control study, 51 total subjects were enrolled: 39 patients diagnosed with sarcoid-related uveitis and 12 patients with systemic sarcoidosis without ocular involvement as controls. Sanger sequencing of the eleven exons of the *NOD2* gene was performed on DNA obtained from whole blood. Sanger sequencing data were aligned against the *NOD2* NCBI-RefSeq reference sequence to identify novel mutations in uveitis patients. For common variants, allele frequencies in cases versus controls were compared using the chi-square test.

Results: There were no significant differences in *NOD2* common variant allele frequencies between sarcoidosis patients with and without uveitis, and none of the pathogenic *NOD2* mutations associated with Blau syndrome were found in this cohort. However, four rare, non-synonymous variants were identified in four patients with ocular sarcoidosis and none of the controls. Variants rs149071116, rs35285618, and 16:g.50745164T > C have never been previously reported to be associated with any disease and may be pathogenic. The fourth variant, rs2066845, is associated with Crohn's disease and psoriatic arthritis.

Conclusions: Despite the phenotypic overlap between sarcoidosis and Blau syndrome, none of the established pathogenic *NOD2* variants were present in adults with sarcoidosis. However, four novel, rare, non-synonymous variants were identified in four cases with ocular sarcoidosis. Further investigation is needed to explore the potential clinical significance of these polymorphisms.

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1. Introduction

NOD2 mutations have been shown to be responsible for three granulomatous inflammatory diseases: Crohn's disease, *NOD2*-associated autoinflammatory disease and Blau syndrome [1]. Blau syndrome is a systemic Mendelian disease whose diagnostic triad includes uveitis, granulomatous dermatitis, and arthritis [1], and its

phenotype overlaps significantly with early-onset sarcoidosis. Because of the similarity between sarcoidosis and Blau syndrome, there is a strong rationale to investigate *NOD2* variation in patients with adult-onset sarcoidosis, particularly those who develop uveitis as this is a characteristic of both disorders. One previous study examined *NOD2* variants in 13 patients with sarcoidosis-associated uveitis [2]. The investigators did not find any of the disease-causing variants associated with Blau syndrome in sarcoidosis patients. The purpose of this study is to investigate *NOD2* variants, including those that have been newly discovered since the previous publication, in a larger sample size of sarcoidosis patients with and without uveitis to determine if there are previously reported mutations or novel mutations in *NOD2* that predispose to ocular involvement.

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2. Materials and methods

Patients with sarcoidosis were identified from the Ocular Immunology and Uveitis Clinics of the Massachusetts Eye and Ear Infirmary (MEEI). This study was approved by the MEEI Institutional Review Board/Human Studies Committee. Controls were defined as patients with systemic sarcoidosis but no ocular involvement. Cases were defined as having sarcoidosis-related uveitis. Systemic sarcoidosis was defined based on definitive or presumed sarcoidosis by American Thoracic Society (ATS) and the World Association for Sarcoidosis and Other Granulomatous Disorders (WASOG) statement criteria [3]. With this criteria, diagnosis is established by either: 1) histological confirmation with evidence of disease involvement in either the thorax or two more other organ systems; or 2) characteristic chest radiographs (bilateral hilar lymphadenopathy) plus erythema nodosum or without diagnosis of another explanatory condition after a minimum of 2 years follow up. Presence or absence of uveitis was determined by clinical examination by a fellowship-trained uveitis specialist. Classification of ophthalmic involvement was based on the criteria set forth by the International Workshop on Ocular Sarcoidosis (IWOS) [4]. A diagnosis of definitive ocular sarcoidosis was made in the presence of a compatible uveitis with a confirmatory biopsy. Presumed ocular sarcoidosis was defined by a compatible uveitis supported by bilateral hilar lymphadenopathy seen on chest imaging without a biopsy.

Sanger sequencing of the eleven exons of the *NOD2* gene were performed on DNA extracted from the participants' blood. Sanger sequencing data were aligned against the *NOD2* NCBI-RefSeq reference sequence. DNASTAR SeqMan Pro software was used for quality control filtering, variant calling and analysis. The variants detected in our patients were cross-referenced against *NOD2* variants that have been previously associated with Blau syndrome, sarcoidosis or Crohn's disease [1]. They were also annotated to identify novel rare, non-synonymous mutations in *NOD2* for potential functional significance using Human BP Codon Resource (HBCR). HBCR uses predicted sequence changes based on Ensembl gene models, and pathogenicity data extracted from a curated database, PolyPhen, Sorting Intolerant From Tolerant (SIFT), the database of Non-Synonymous Functional Prediction (dbNSFP) resource, and Combined Annotation Dependent Depletion (CADD) scores. HBCR also annotates variants with allele frequencies in multiple reference populations.

Baseline demographic and clinical characteristics between cases and controls were compared using the *t*-test for continuous variables and the chi-square test for categorical variables. For common *NOD2* variants, allele frequencies between cases and control were compared using the chi-square test. All statistical analyses were performed in Stata 12.1 (College Station, Texas).

3. Results

51 subjects were enrolled, including 39 patients diagnosed with sarcoid-related uveitis as cases and 12 patients with systemic sarcoidosis without ocular involvement as controls. Table 1 summarizes the clinical characteristics of the patients. The gender, age, race and duration of sarcoidosis were not statistically different between cases and controls ($p > 0.05$).

In our patients with adult-onset sarcoidosis, we did not identify any of the single amino acid mutations in *NOD2* that have been associated with Blau syndrome [1,5]. Table 2 shows all of the polymorphisms in *NOD2* identified by sequencing in our patients. In comparing with the previous publication on *NOD2* variants in ocular sarcoidosis, there were six variants that led to protein changes that overlapped between the two studies. Four of them were common variants (rs2067085, rs2066842, rs2066843, rs1861759) and we did not find that they were associated with uveitis to a statistically significant degree. The fifth was a rare variant associated with Crohn's disease (rs2066844, MAF = 0.03). In our cohort, none of the patients with this variant had a history of Crohn's disease. The sixth (rs5743291) was a rare variant associated with early onset sarcoidosis, but the frequency of this allele was not significantly different between those with and without uveitis [minor allele frequencies (MAFs) = 0.067 and 0.068 respectively].

We identified four rare, non-synonymous variants that were present in only one case each. Two, rs149071116 and novel variant NM_022162.1:c.1342T > C (*NOD2*:p.Ser448Pro), have never been previously associated with any disease. SIFT, a sequence homology-based tool that sorts intolerant from tolerant amino acid substitutions and predicts whether an amino acid substitution will have a phenotypic effect, predicted both variants to be damaging. Polymorphism rs149071116 was identified in a 34-year-old Asian man with anterior uveitis in the right eye and panuveitis and macular edema in the left eye. The patient with variant NM_022162.1:c.1342T > C (*NOD2*:p.Ser448Pro) was a 36-year-old Caucasian man with panuveitis, choroiditis, periphlebitis and papillitis. He required methotrexate in addition to his prednisone to control his ocular inflammation and also had renal and pulmonary involvement during course of his disease. The third polymorphism, rs35285618, is a low frequency single nucleotide protein (SNP) (MAF = 0.013) located six amino acids downstream of the R702W variant that is associated with Crohn's disease in African Americans [6]. It was predicted to be "possibly damaging" by PolyPhen but "tolerated" by SIFT. It was found in a 40-year old Hispanic man of European descent with bilateral panuveitis, lung, skin, and parotid gland involvement. The fourth polymorphism, rs2066845, is also a low frequency SNP that is associated with Crohn's disease and psoriatic arthritis [7–9]. The patient with this variant was a 64-year-

Table 1
Demographic and clinical characteristics of sarcoidosis patients.

		Sarcoidosis with uveitis (n = 39)	Sarcoidosis without uveitis (n = 12)	P value ^a
Age in years, mean (SD)		56.6 (15.1)	54.2 (6.8)	0.58
Gender	M	11	5	0.48
	F	28	7	
Duration of sarcoidosis in years, mean (SD)		9.1 (7.5)	8.3 (10.3)	0.72
Ethnicity	CA	28	10	0.27
	AA	10	1	
	HA	1	1	

SD = standard deviation, M = male, F = female, CA = Caucasian American, AA = African American, HA = Hispanic American.

^a Continuous variables compared with the *t*-test and categorical variables compared with the chi-square test.

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