Genetic Characterization and Susceptibility for Sarcoidosis in Japanese Patients: Risk Factors of *BTNL2* Gene Polymorphisms and HLA Class II Alleles

Hitomi Suzuki,[1–3] *Masao Ota,*[3,4] *Akira Meguro,*[1] *Yoshihiko Katsuyama,*[5] *Tatukata Kawagoe,*[1] *Mami Ishihara,*[1,6] *Yuri Asukata,*[1] *Masaki Takeuchi,*[1] *Norihiko Ito,*[1] *Etsuko Shibuya,*[1] *Eiichi Nomura,*[1] *Riyo Uemoto,*[1] *Tadayuki Nishide,*[1] *Kenichi Namba,*[7] *Nobuyoshi Kitaichi,*[8] *Shin-ichiro Morimoto,*[9] *Toshikatsu Kaburaki,*[10] *Yasutaka Ando,*[11] *Shinobu Takenaka,*[12] *Jutaro Nakamura,*[1] *Kozou Saeki,*[2] *Shigeaki Ohno,*[7] *Hidetoshi Inoko,*[13] *and Nobuhisa Mizuki*[1]

Purpose. Sarcoidosis is a heterogeneous and multisystem granulomatous disorder. The etiology still is uncertain, but the disease currently is thought to be triggered by various genetic as well as environmental factors. Recently, an association between sarcoidosis and the *butyrophilin-like 2* (*BTNL2*) gene located in close proximity to the *HLA-DRB1* gene was reported. The purpose of our study was to verify the relationship between *BTNL2* and *HLA* risk alleles for the susceptibility to sarcoidosis, and to assess whether the *BTNL2* association is independent of the *HLA* risk alleles.

Methods. In our study, 11 single nucleotide polymorphisms (rs28362677, rs2076533, rs2076530, rs2076529, rs2294881, rs3763304, rs2076523, rs28362682, rs3806156, rs9268499, rs3763317), including the functional rs2076530 (G > A) of the *BTNL2* gene, and *HLA-DRB1* and *-DQB1* alleles, were genotyped in 237 Japanese patients diagnosed with sarcoidosis and 287 healthy Japanese control subjects.

Results. In the patient group, the *HLA-DRB1*08:03* ($P = 6.15 \times 10^{-5}$, odds ratio [OR] = 2.43) and *BTNL2* rs2076530_A ($P = 6.90 \times 10^{-6}$, OR = 1.84) were associated with disease susceptibility. Upon stratification analysis in search for a synergistic effect given the extensive linkage disequilibrium between *BTNL2* rs2076530_A and *HLA-DRB1*08:03*, our results suggested that the risk-bearing allele of these two loci interact negatively. No significant differences were observed in allele frequencies for alleles in patients with ocular and other systemic sarcoidosis.

Conclusions. Our studies implicated that the *HLA-DRB1* allele is a major contributing genetic factor in the development of sarcoidosis in Japan. However, further studies are needed to verify how *HLA* or *BTNL2* alleles confer the disease phenotype, severity of sarcoidosis. (*Invest Ophthalmol Vis Sci.* 2012;53:7109–7115) DOI:10.1167/iovs.12-10491

From the [1]Department of Ophthalmology and Visual Science, Yokohama City University Graduate School of Medicine, Kanagawa, Japan; [2]Saeki Eye Clinic, Odawara, Japan; the Departments of [4]Legal Medicine and [5]Pharmacy, Shinshu University School of Medicine, Nagano, Japan; the [6]Hiyoshi Eye Clinic, Yokohama, Japan; the [7]Department of Ophthalmology and Visual Sciences, Hokkaido University Graduate School of Medicine, Hokkaido, Japan; the [8]Department of Ophthalmology, Health Sciences University of Hokkaido, Sapporo, Japan; the [9]Division of Cardiology, Department of Internal Medicine, Fujita Health University School of Medicine, Toyoake, Aichi, Japan; the [10]Department of Ophthalmology, University of Tokyo School of Medicine, Tokyo, Japan; the [11]Department of Ophthalmology, Kitasato Institute Hospital, Tokyo, Japan; the [12]Department of Respiratory Diseases, Kumamoto City Hospital, Kumamoto, Japan; and the [13]Department of Genetic Information, Division of Molecular Life Science, Tokai University School of Medicine, Kanagawa, Japan.

[3]These authors contributed equally to this work presented here and therefore should be regarded as equivalent authors.

Supported by Grant-in-Aid for the National Cerebral and Cardiovascular Center (ID:20-5) from the Ministry of Health, Labour and Welfare of Japan.

Submitted for publication June 29, 2012; revised August 16 and August 30, 2012; accepted September 12, 2012.

Disclosure: **H. Suzuki**, None; **M. Ota**, None; **A. Meguro**, None; **Y. Katsuyama**, None; **T. Kawagoe**, None; **M. Ishihara**, None; **Y. Asukata**, None; **M. Takeuchi**, None; **N. Ito**, None; **E. Shibuya**, None; **E. Nomura**, None; **R. Uemoto**, None; **T. Nishide**, None; **K. Namba**, None; **N. Kitaichi**, None; **S. Morimoto**, None; **T. Kaburaki**, None; **Y. Ando**, None; **S. Takenaka**, None; **J. Nakamura**, None; **K. Saeki**, None; **S. Ohno**, None; **H. Inoko**, None; **N. Mizuki**, None

Corresponding author: Masao Ota, Department of Legal Medicine, Shinshu University School of Medicine, Nagano, Japan; otamasao@shinshu-u.ac.jp.

Sarcoidosis is a chronic systemic disease characterized by marked macrophage and CD4+ T-cell activity, and the accumulation of noncaseating granulomas in a wide range of organs, such as lungs, lymph nodes, eye, skin, and heart.[1] In the current survey of uveitis in Japan, the most frequent inflammatory disease was sarcoidosis (10.6%), followed by Vogt-Koyanagi-Harada disease (7.0%), acute anterior uveitis (6.5%), scleritis (6.1%), herpetic iridocyclitis (4.2%), Behçet's disease (3.9%), bacterial endophthalmitis (2.5%), masquerade syndrome (2.5%), Posner-Schlossman syndrome (1.8%), and retinal vasculitis (1.6%).[2] The etiology of sarcoidosis still is uncertain, but the disease currently is thought to be triggered by various genetic as well as environmental factors. It is well established that sarcoidosis is associated with the human leukocyte antigen (HLA) class II genes, especially *HLA-DRB1* and *HLA-DQB1* genes, in several ethnic groups.[3–7] However, to our knowledge it has not yet been clarified whether HLA genes themselves are the pathogenic gene related directly to sarcoidosis or are associated with the disease only because of linkage disequilibrium with some other genes. Recently, a single nucleotide polymorphism within *butyrophilin-like 2* (*BTNL2*), rs2076530, has been implicated as a risk factor for sarcoidosis.[8] The *BTNL2* gene, located only 170 kilobases (kb) from the *HLA-DRB1* gene telomerically on chromosome 6, is a member of the immunoglobulin superfamily with likely costimulatory activities in T-cell

activation on the basis of amino acid homology to B7 molecules.[9] The G/A transition of rs2076530 causes a premature truncation of protein, disrupting the membrane localization of the protein and a necessary process for down-regulating activated T-cells (Th1).[8,10] The truncated protein increases the risk of developing sarcoidosis independent of *HLA-DRB1* risk alleles.[8,11] However, subsequent studies have showed inconsistent results on a sarcoidosis risk factor of *BTNL2* rs2076530_A and its independent effect of *HLA-DRB1*.[12,13] In other diseases, such as type 1 diabetes (T1D),[14] rheumatoid arthritis (RA),[15] systemic lupus erythematosus (SLE),[14] multiple sclerosis (MS),[15] Graves' disease (GD),[16] chronic beryllium disease (CBD),[17] and ulcerative colitis (UC),[18] a significant association with *BTNL2* polymorphisms appeared to be secondary to the primary *HLA-DRB1* association because of its strong linkage disequilibrium (LD) with *DRB1* alleles.

In our study, to verify the association between *BTNL2* and sarcoidosis, and independence from the *HLA-DRB1* risk alleles, we performed a case-control association study of the *BTNL2* polymorphisms and *HLA-DRB1* alleles in Japanese sarcoidosis patients.

Materials and Methods

Subjects

The total subject group consisted of 239 Japanese patients with sarcoidosis and 287 healthy Japanese controls from Yokohama City University, Hokkaido University, Fujita Health University, Tokyo University, Keio University, the Kumamoto City hospital, and the Yuasa Eye Clinic. All 239 patients had chronic sarcoidosis, which was diagnosed according to the "diagnostic criteria and guidelines for sarcoidosis" developed by the Japanese Society of Sarcoidosis and Other Granulomatous Disorders (JSSOG 2007).[19] The diagnostic criteria used currently in Japan include histologic and clinical diagnosis. The key criteria are clinical features from laboratory examination, radiologic features, pathologic findings (noncaseous epithelioid cell granuloma in biopsy specimens), and exclusion of other diseases. The concepts agree with the ATS/ERS/WASOG statement on sarcoidosis (ATS/ERS/WASOG 1999).[20] A clinical diagnostic group presenting no histologic evidence of granuloma, but demonstrating clinical features suggesting sarcoid lesions in two or more organs, and supported by the evidence from two or more characteristic clinical and radiologic features include bilateral hilar lymphadenopathy (BHL) on chest x-ray and/or findings of diagnostic imaging, such as CT/HRCT or bronchoscopy, elevated serum ACE, negative tuberculin test, abnormal uptake on Gallium-67 citrate scintigraphy, and an increase in lymphocyte count or an elevated CD4/CD8 ratio in bronchoalveolar lavage fluid.

Among the patients with sarcoidosis, 198 had ocular (uveitis), 138 lung, 47 skin, 48 cardiac, and 10 nerve lesions (Table 1). All control subjects were unrelated healthy volunteers who were ethnically similar to the patients. Details of the study were explained to all patients and controls, and written informed consent was obtained for genetic screening. The study obtained the approval of the Ethics Committee of Yokohama City University School of Medicine, and was in compliance with the guidelines of the Declaration of Helsinki.

Genomic DNA was extracted from peripheral blood cells using the QIAamp DNA Blood Maxi Kit (Qiagen, Tokyo, Japan).

Genotyping of Single-Nucleotide Polymorphisms (SNPs) in *BTNL2* Region

We examined 11 SNPs: rs3763317, rs9268499, rs3806156, rs28362682, rs2076523, rs3763304, rs2294881, rs2076529, rs2076530, rs2076533, and rs28362677 selected based on information from public sources, including the NCBI dbSNP, ABI, and HapMap databases, and a previous report.[8] Genotyping of all SNPs was performed by the TaqMan 5′ exonuclease assay using primers supplied by ABI (Foster City, CA).

Table 1. Characteristics and Frequencies of Clinical Phenotype

	Sarcoidosis	Controls
N	239	287
Age at onset, mean	49.6 ± 16.2	33.1 ± 9.4
Sex, men/women	0.245/0.755	0.456/0.544
	n	Frequencies
Diagnostic criteria		
Clinical	135	0.565
Histologic	104	0.435
Lesions		
Lung	138	0.577
Ocular, uveitis	198	0.828
Skin	47	0.197
Heart	48	0.201
Nerve	10	0.042
Chronic	239	1.000
Lofgren syndrome	0	0.000

HLA-DRB1 and *HLA-DQB1* Genotyping

Genotyping of *HLA-DRB1* and *-DQB1* alleles was performed by Luminex reverse sequence-specific oligonucleotides using bead kits from One Lambda, Inc. (Canoga Park, CA). Additional PCR sequence-specific primer of SBT reactions were used to determine high-resolution alleles, if necessary.

Statistical Analysis

Allele frequencies of all detected SNPs were tested for Hardy-Weinberg equilibrium (HWE). Differences in allele frequencies between cases and controls were assessed with the χ^2 test or Fisher's exact test. The Haploview 3.32 program was used to compute pairwise LD statistics.[21] Standardized disequilibrium D' and r^2 were calculated. LD blocks were defined according to the criteria of Gabriel et al.[22] Haplotype frequencies were estimated with an accelerated expectation-maximization algorithm, similar to the partition–ligation–expectation–maximization method described previously.[23] All P values were derived from a 2-sided test, and P values < 0.05 were considered statistically significant. To obtain a measure of significance corrected for multiple testing we applied Bonferroni's correction.

Results

The Case-Control Association Study of *BTNL2* Polymorphisms

Eleven biallelic SNPs in the *BTNL2* gene were genotyped in all the sarcoidosis patients and control subjects (Fig. 1). Among 11 SNPs, the SNP 9 (rs2076530)_A allele showed extremely strong association with susceptibility to the disease ($P = 6.9 \times 10^{-6}$, odds ratio [OR] = 1.84, Table 2). All 11 SNPs were in HWE between cases and controls.

The haplotype frequencies and structures were estimated and constructed from the 11 SNPs using the Haploview software (Table 3). Three LD blocks were induced (Fig. 2) and no significant differences were observed in the haplotype frequencies of each block between patients and controls groups. However, the haplotype distributions in blocks 2 and 3 were different between the *DRB1*08:03*-positive patient group and the all patients group, and also the control group. The third frequent haplotype of block 2 in the control and all

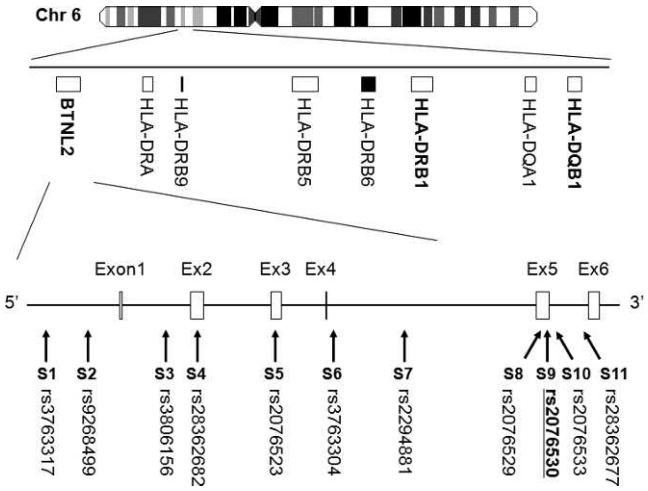

Figure 1. Location of 11 SNPs in the *BTNL2* used in our study and genomic organization of 257 kb of the MHC region of human chromosome 6, at 6p21.31, showing the relative location of *BTNL2* and HLA-Class II genes.

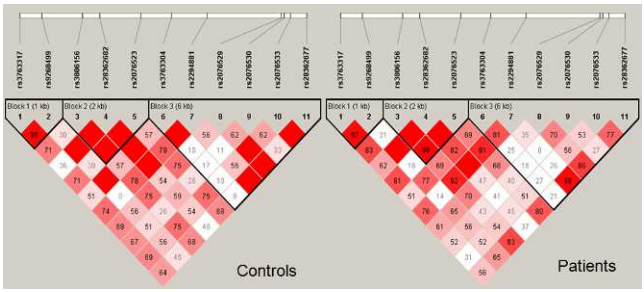

Figure 2. Structure of LD plotted for 11 SNPs in the *BTNL2*. The D' values corresponding to each SNP pair are expressed as a percentage and shown within the respective square. The haplotype blocks were determined using the Haploview 4.2 software. The gradient of D' value (low to high value) is indicated by different shades of *red* (*light* to *dark*, respectively).

patients groups, A–A–G was the most frequent haplotype in *DRB1*08:03*-positive patients (57.9%), and the second most frequent haplotype of block 3, A–G–A–A–G–G was the most frequent haplotype in *DRB1*08:03*-positive patients (53.6%).

Association Analysis of *HLA-DRB1* and *HLA-DQB1* Alleles

To determine whether there were different allelic distributions in sarcoidosis patients and controls, we performed an association test for each allele group of the *DRB1* and *DQB1* alleles. The frequency of the *HLA-DRB1*08:03* allele was significantly different between sarcoidosis patients and their matched controls (Table 4, $P = 6.90 \times 10^{-6}$, OR = 2.43). Among DRB1 alleles, two risk alleles, *DRB1*08:03* and *DRB1*09:01*, were observed in the disease susceptibility. No statistically significant differences were detected between cases and controls of *DQB1* alleles.

LD Analysis between *BTNL2* and *DRB1* Alleles

Examination of haplotype frequency between *DRB1–BTNL2* also revealed that the patient group was significantly higher than the control group at *rs2076530_A* and *DRB1*08:03* haplotype ($P = 7.72 \times 10^{-6}$, OR = 2.42). Almost 85% were *rs2076530_A* propositi in the *DRB1*08:03*-positive patient groups.

The haplotype *rs2076530_A* and *DRB1*09:01* showed weaker association with the disease susceptibility ($P = 0.005$) than the *rs2076530_A* and *DRB1*08:03* haplotype (Table 5). D' (degree of LD) value between *DRB1*08:03* or *DRB1*09:01* and *rs2076530_A* was 0.92 and 0.84 in patients, and 0.90 and 0.83 in controls, respectively.

We investigated whether these two loci contributed independently to susceptibility for sarcoidosis or the possible confounding effect is related. In this analysis, we stratified the study cohort into *HLA-DRB1* risk alleles (*DRB1*08:03* and *DRB1*09:01*) carriers and *HLA-DRB1* risk alleles noncarriers (Table 6). No significant association with the disease was observed for rs2076530_A in noncarriers of *DRB1* risk alleles. However, for the carriers of *DRB1* risk alleles, the *BTNL2* risk allele showed weak association with sarcoidosis ($P = 0.011$). Meanwhile, for the carriers of the rs2076530_A allele, *HLA-DRB1* risk alleles showed an extremely strong association with sarcoidosis.

We also carried out two-locus analyses to detect the alleles that showed the strongest association among the neighboring loci (Table 7), according to the method of Svejgaard and Ryder.[24] Tests 1 and 2 investigated whether the *DRB1** risk alleles are deviating in the *rs2076530_A* positives and negatives. Conversely, the *rs2076530_A* was tested for independent association against the *DRB1** risk alleles in tests 3 and 4 in the presence and absence of *DRB1** risk alleles, respectively. In test 7, it was investigated whether the *DRB1**

Table 2. Allele Frequencies of SNPs of the *BTNL2* Gene between Sarcoidosis Patients and Controls

SNP			Risk	Risk Allele Frequency, *n* (%)				
Name	db SNP	Allele	Allele	Cases ($n = 237$)	Controls ($n = 287$)	*P*	*Pc*	OR (95% CI)
S1	rs3763317	A/G	G	162 (34.2)	162 (28.2)	0.038		
S2	rs9268499	C/T	C	274 (57.8)	288 (50.2)	0.014		
S3	rs3806156	A/C	A	267 (56.3)	314 (54.7)			
S4	rs28362682	T/A	A	121 (25.5)	113 (19.7)	0.024		
S5	rs2076523	G/A	G	267 (56.3)	314 (54.7)			
S6	rs3763304	G/A	A	129 (27.2)	142 (24.7)			
S7	rs2294881	G/A	A	243 (51.3)	285 (49.7)			
S8	rs2076529	A/G	A	299 (63.1)	322 (56.1)	0.022		
S9	rs2076530	A/G	A	355 (74.9)	355 (61.8)	0.0000069	0.0003	1.84 (1.41–2.40)
S10	rs2076533	G/A	G	298 (62.9)	322 (56.1)	0.026		
S11	rs28362677	G/A	G	342 (72.2)	376 (65.5)	0.021		

CI, confidence intervals.

Table 3. Haplotye Structure and Frequency in Three Blocks of BTNL2 Observed in Controls, Patients, and *DRB1*08:03*-Positive Patients

	Block 1			Block 2				Block 3						
	SNP1	SNP2	Freq.	SNP3	SNP4	SNP5	Freq.	SNP6	SNP7	SNP8	SNP9	SNP10	SNP11	Freq.
Controls, $n = 287$	A	T	0.496	C	T	A	0.453	G	A	A	A	G	G	0.329
	G	C	0.280	A	T	G	0.256	A	G	A	A	G	G	0.159
	A	C	0.222	A	A	G	0.197	G	G	G	A	A	A	0.131
								G	G	G	G	A	A	0.125
								G	A	G	G	A	G	0.094
								A	G	G	G	A	A	0.089
								G	A	A	G	G	G	0.073
Patients, $n = 239$	A	T	0.419	C	T	A	0.435	G	A	A	A	G	G	0.298
	G	C	0.339	A	T	G	0.308	A	G	A	A	G	G	0.165
	A	C	0.239	A	A	G	0.253	G	G	G	A	A	A	0.108
								G	G	G	G	A	A	0.064
								G	A	G	G	A	G	0.049
								G	A	A	A	A	G	0.046
								A	G	G	G	A	A	0.045
*DRB1*08:03*-Positive Patients, $n = 96$	A	T	0.469	A	A	G	0.579	A	G	A	A	G	G	0.536
	G	C	0.280	A	T	G	0.221	G	G	G	A	A	A	0.171
	A	C	0.222	C	T	A	0.200	G	A	A	A	G	G	0.143
								A	G	G	G	A	A	0.079
								G	A	G	G	A	G	0.050
								G	A	A	G	G	G	0.021

Freq., frequency.

risk alleles and *rs2076530_A* association differ, and in test 8 the combined association of the *DRB1** risk alleles and *rs2076530_A* was compared relative to their absence.

As shown in Table 7, the *DRB1** risk alleles contributed to the susceptibility independently of the *rs2076530_A* (test 1), whereas the *rs2076530_A* showed no significant association in *DRB1** risk alleles –positives (test 3) or –negatives (test 4). The synergistic effect between two alleles was not observed (test 6), because the OR (2.39) for persons with both alleles was not exactly higher than a risk allele at one locus (Tables 2 and 4, OR was 2.43 for the *DRB1*08:03* and 1.84 for the *rs2076530_A*).

Discussion

SNPs in the *BTNL2* gene have been reported to be associated with the disease susceptibility for sarcoidosis.[8,11,25] Especially, a functional *BTNL2* polymorphism rs2076530_A allele has been discussed on disease susceptibility conferred dependently or independently with *HLA-DRB1* alleles.[8,11–13] This reflects the extensive linkage disequilibrium across the HLA region and the difficulties to assign primary associated locus within this genomic region.[14–16] Multivariable logistic regression analyses showed that *BTNL2* effects are independent of human leukocyte antigen class II genes in Caucasians, but may interact antagonistically in African Americans.[11] In our study, strong LD was observed between *DRB1** alleles (*DRB1*08, DRB1*09, DRB1*11, DRB1*12, DRB1*14, DRB1*15*) and *BTNL2 rs2076530_A,* particularly for *DRB1*08:03* or the *DRB1*09:01* allele and *BTNL2 rs2076530_A*. Haplotype structures and frequencies in the *BTNL2* gene were not different between the patient and control groups. In contrast, patients carrying *DRB1*0803* showed different structure and frequencies of haplotypes in blocks 2 and 3. These haplotypes were specific for the *DRB1*0803* allele due to strong LD. Taken together, our findings supported no evidence of an independent genetic association between BTNL2 polymorphisms rs2076530_A and the susceptibility to sarcoidosis.

Numerous studies have suggested that there is an association between certain HLA alleles and sarcoidosis.[26–30] However the susceptible allele was different (*DRB1*03*, *DRB1*11*, *DRB1*12*, *DRB1*14*, and *DRB1*15* on *HLA class II* gene) in various countries.[4–7,27,31] This may be influenced by different diagnosis and clinical characteristics of the study subjects, and the ethnicity studied. No consensus was established about which HLA locus is involved directly in the pathogenesis of sarcoidosis, but the most recent studies focused mainly on the *HLA-DRB1* genes. It is probable to suppose that the *HLA-DRB1* alleles have been the best studied candidate genes in sarcoidosis because DR proteins are responsible for the presentation of specific antigens that are able to start and maintain the immunopathologic process. The loading of a particular peptide onto an HLA class II molecule is dependent strictly on the characteristic of the peptide-binding groove of the molecules.[32–34] In HLA-DR molecules, pockets P1, P4, P6, P7, and P9 are present within the binding groove.[33] Among the 5 pockets, the amino acid epitopes in pockets 4, 6, and 7 seem to have a role in the susceptibility of sarcoidosis.[4,5,28,35] The combination of specific amino acids residues at 11, 47, and 71 positions in three pockets appears to be implicated in susceptible or protective determinants for disease progression. The risk allele *DRB1*08:03* in Japanese patients with sarcoidosis carries *HLA-DRB1*-S[11], *HLA-DRB1*-Y[47], and *HLA-DRB1*-R[71].

These associations also could be involved in the specific forms,[35,36] clinical features,[37,38] progression,[39,40] and prognosis[41] of the disease. Our data clearly showed the existence of an association between *HLA-DRB1*08:03* and sarcoidosis in general (regardless of the onset of the disease and subtypes of the disease).

BTNL2 is a member of the immunoglobulin superfamily and a role as co-stimulatory receptor involved in modulation of T-cell response on the basis of the amino acid homology of B7 (CD80 and CD86) proteins by experiments in mice.[10] A possible modulatory role for the protein in intestine inflammation has been proposed in this animal model. However, the function of human BTNL2 differs significantly from that of its

Table 4. Distribution of *HLA-DRB1* and *-DQB1* Alleles in Patients with Sarcoidosis and in Healthy Control Subjects

	Frequency, *n* (%)					
	Cases ($n = 237$)		Controls ($n = 287$)		*P*	OR (95% CI)
*HLA-DRB1**						
01:01	8 (1.7)		29 (5.1)		0.0037	0.32 (0.14–0.70)
03:01	0 (0.0)		2 (0.4)			
04:01	4 (0.8)		1 (0.2)			
04:03	13 (2.7)		18 (3.2)			
04:05	63 (13.3)		73 (12.9)			
04:06	15 (3.2)		20 (3.5)			
04:07	1 (0.2)		3 (0.5)			
04:09	1 (0.2)		0 (0.0)			
04:10	8 (1.7)		9 (1.6)			
08:02	17 (3.6)		34 (6.0)			
08:03	78 (16.5)		43 (7.6)		6.9E-06	2.43 (1.64–3.61)
08:09	1 (0.2)		0 (0.0)			
09:01	99 (20.9)		80 (14.1)		0.0039	1.61 (1.16–2.23)
11:01	7 (1.5)		11 (1.9)			
12:01	24 (5.1)		18 (3.2)			
12:02	9 (1.9)		10 (1.8)			
13:01	3 (0.6)		2 (0.4)			
13:02	15 (3.2)		42 (7.4)		0.0026	0.41 (0.22–0.75)
13:03	0 (0.0)		1 (0.2)			
13:07	0 (0.0)		1 (0.2)			
14:03	9 (1.9)		6 (1.1)			
14:05	5 (1.1)		11 (1.9)			
14:06	2 (0.4)		12 (2.1)		0.027	0.20 (0.04–0.88)
14:07	3 (0.6)		0 (0.0)			
14:09	0 (0.0)		2 (0.4)			
14:12	1 (0.2)		1 (0.2)			
14:54	20 (4.2)		22 (3.9)			
15:01	25 (5.3)		37 (6.5)			
15:02	43 (9.1)		75 (13.2)		0.039	0.66 (0.44–0.97)
16:02	0 (0.0)		5 (0.9)			
HLA-DQB1						
*DQB1*0201*	0	0.0	2	0.4		
*DQB1*0301*	53	11.2	60	10.8		
*DQB1*0302*	43	9.1	62	11.2		
*DQB1*0303*	101	21.3	82	14.7	0.0069	1.57 (1.14–2.16)
*DQB1*0401*	59	12.4	67	12.1		
*DQB1*0402*	21	4.4	26	4.7		
*DQB1*0501*	8	1.7	30	5.4	0.0015	0.30 (0.14–0.66)
*DQB1*0502*	6	1.3	15	2.7		
*DQB1*0503*	22	4.6	25	4.5		
*DQB1*0601*	119	25.1	115	20.7		
*DQB1*0602*	23	4.9	31	5.6		
*DQB1*0603*	3	0.6	1	0.2		
*DQB1*0604*	15	3.2	40	7.2	0.0050	0.42 (0.23–0.77)
*DQB1*0622*	1	0.2	0	0.0		

Table 5. Linkage Disequilibrium between *BTNL2 rs2076530_A* and *HLA-DRB1*08:03* or *-DRB1*09:01*

		rs2076530_A (SNP9)	
Samples	***HLA-DRB1***	***D'***	r^2
Total, $n = 547$	**08:03*	0.92	0.05
	**09:01*	0.84	0.07
Controls, $n = 287$	**08:03*	0.90	0.04
	**09:01*	0.83	0.06
Patients, $n = 237$	**08:03*	0.92	0.05
	**09:01*	0.84	0.06

murine ortholog in a number of respects.[8,10,42] In addition, sarcoidosis, tuberculoid leprosy, tuberculosis, and Crohn's disease are Th1-mediated diseases characterized by granuloma formation. In Th1-dominated granulomatous diseases, the truncating BTNL2 SNP (rs2076530) was not supported to be important.[43]

Therefore, to our knowledge the physiologic role of human BTNBL2 has not yet been elucidated, and the role of the protein in the pathogenesis of autoimmune diseases remains to be revealed. Taken together, we concluded that there probably is no major role for BTNL2 in the pathogenesis of sarcoidosis from our results presented.

Table 6. Effects of LD between *DRB1* Risk Alleles and *rs2076530_A* Allele on Association Results

	Patients		Controls		*P*	OR (95% CI)
Stratified with *HLA-DRB1* risk alleles						
DRB1 risk alleles* rs2076530_A frequency, *n* (%)						
Carrier	$n = 134$	239 (89.2)	$n = 111$	180 (81.1)	0.01	1.92 (1.15–3.21)
Noncarrier	$n = 103$	116 (56.3)	$n = 176$	175 (49.7)	0.13	1.30 (0.92–1.84)
Stratified with *rs2076530_A* allele						
rs2076530_A DRB1 risk alleles* frequency, *n* (%)						
Carrier	$n = 199$	173 (43.5)	$n = 217$	120 (27.6)	1.83×10^{-6}	2.01 (1.51–2.69)
Noncarrier	$n = 38$	4 (5.3)	$n = 70$	3 (2.1)	0.22	2.54 (0.55–11.65)

* *DRB1*08:03* and *DRB1*09:01*.

Table 7. Two-Locus Analysis of Genetic Risk of *DRB1* Risk Alleles and *rs2076530_A* Allele to Sarcoidosis

DRB1 Risk Alleles*	rs2076530_A Allele	Cases, $n = 237$	Controls, $n = 287$
Observed data			
+	+	130	109
+	−	4	2
−	+	69	108
−	−	34	68

		OR	*P* Value
Two–by–two comparisons (DRB1 risk alleles and rs2076530_A allele)			
T1	++ vs. −+	1.87	0.002
T2	+− vs. −−	3.13	0.222
T3	++ vs. +−	0.60	0.856
T4	−+ vs. −−	1.28	0.346
T5	+− vs. −+	4.00	0.348
T6	++ vs. −−	2.39	0.000037

T1 and T2, tests for independent DRB1 risk alleles; T3 and T4, tests for independent *rs2076530_A* allele; T5, tests for difference between DRB1 risk alleles and *rs2076530_A* allele to sarcoidosis; T6, combined association.

* *DRB1*08:03* and *DRB1*09:01*.

Recent studies have highlighted the effecter role of Th17 cells in pathologic conditions, including autoimmunity and inflammation.[44] An elevation of Th17 cells was demonstrated in the peripheral blood and bronchoalveolar lavage of patients with active sarcoidosis.[45] Restrict inflammation caused by a joint Th1/Th2 response might be involved in the pathogenesis of granuloma formation in sarcoidosis.

Accurate clinical diagnosis, especially where disease heterogeneity is known to exist, large sample sizes, and the precise mapping of the HLA region will provide additional power to dissect gene effects in determining susceptibility to sarcoidosis.

References

1. Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. *N Engl J Med*. 2007;357:2153–2165.
2. Ohguro N, Sonoda K, Takeuchi M, Matsumara M, Mochizuki M. The 2009 prospective multi-center epidemiologic survey of uveitis in Japan. *Jpn J Ophtalmol*. 2012;56:432–435.
3. Ishihara M, Ishida T, Inoko H, et al. HLA serological and class II genotyping in sarcoidosis patients in Japan. *Jpn J Ophthalmol*. 1996;40:86–94.
4. Foley PJ, McGrath DS, Puscinska E, et al. Human leukocyte antigen-DRB1 position 11 residues are a common protective marker for sarcoidosis. *Am J Respir Cell Mol Biol*. 2001;25:272–277.
5. Rossman MD, Thompson B, Frederick M, et al. HLA-DRB1*1101: a significant risk factor for sarcoidosis in blacks and whites. *Am J Hum Genet*. 2003;73:720–735.
6. Rybicki BA, Maliarik MJ, Poisson LM, et al. The major histocompatibility complex gene region and sarcoidosis susceptibility in African Americans. *Am J Respir Crit Care Med*. 2003;167:444–449.
7. Sato H, Woodhead FA, Ahmad T, et al. Sarcoidosis HLA class II genotyping distinguishes differences of clinical phenotype across ethnic groups. *Hum Mol Genet*. 2010;19:4100–4111.
8. Valentonyte R, Hampe J, Huse K, et al. Sarcoidosis is associated with a truncating splice site mutation in BTNL2. *Nat Genet*. 2005;37:357–364.
9. Rhodes DA, Stammers M, Malcherek G, Beck S, Trowsdale J. The cluster of BTN genes in the extended major histocompatibility complex. *Genomics*. 2001;71:351–362.
10. Nguyen T, Liu XK, Zhang Y, Dong C. BTNL2, a butyrophilin-like molecule that functions to inhibit T cell activation. *J Immunol*. 2006;176:7354–7360.
11. Rybicki BA, Walewski JL, Maliarik MJ, Kian H, Iannuzzi MC, Access Research Group. The *BTNL2* gene and sarcoidosis susceptibility in African Americans and Whites. *Am J Hum Genet*. 2005;77:491–499.
12. Spagnolo P, Sato H, Grutters JC, et al. Analysis of *BTNL2* genetic polymorphisms in British and Dutch patients with sarcoidosis. *Tissue Antigens*. 2007;70:219–227.
13. Wijnen PA, Voorter CE, Nelemans PJ, Verschakelen JA, Bekers O, Drent M. Butyrophilin-like 2 in pulmonary sarcoidosis: a factor for susceptibility and progression? *Hum Immunol*. 2011;72:342–347.
14. Orozoco G, Eerligh P, Sanchez E, et al. Analysis of a functional *BTNL2* polymorphism in type I diabetes, rheumatoid arthritis, and systemic lupus erythematosus. *Hum Immunol*. 2005;66:1235–1241.
15. Traherne JA, Barcellos LF, Sawcer SJ, et al. Association of the truncating splice site mutation in *BTNL2* with multiple sclerosis is secondary to *HLA-DRB1*15*. *Hum Mol Genet*. 2006;15:155–161.
16. Simmonds MJ, Heward JM, Barrett JC, Franklyn JA, Gough SC. Association of the *BTNL2* rs2076530 single nucleotide polymorphisms with Graves' disease appears to be secondary to *DRB1* exon 2 position β74. *Clin Endocrinol*. 2006;65:429–432.
17. Sato H, Spagnolo P, Silveria L, et al. *BTNL2* allele associations with chronic beryllium disease in HLA-DPB1*Glu69-negative individuals. *Tissue Antigens*. 2007;70:480–486.

18. Mochida A, Kinouchi Y, Negoro K, et al. Butyrophilin-like 2 gene is associated with ulcerative colitis in the Japanese under strong linkage disequilibrium with HLA-DRB1*1502. *Tissue Antigens*. 2007;70:128–135.
19. The Japanese Society of Sarcoidosis and Other Granulomatous Disorders (JSSOG). Diagnostic criteria and guidelines for sarcoidosis-2006. *Jpn J Sarcoidosis and Granulomatous Disorders*. 2007;22:89–101.
20. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. *Am J Respir Crit Care Med*. 1999;160:736–755.
21. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics*. 2005; 21:263–265.
22. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. *Science*. 2002;296: 2225–2229.
23. Qin ZS, Niu T, Liu J. Partition-ligation-expectation-maximization algorithm for haplotype inference with single-nucleotide polymorphisms. *Am J Hum Genet*. 2002;71:1242–1247.
24. Svejgaard A, Ryder LP. HLA and disease associations: detecting the strongest association. *Tissue Antigens*. 1994;43:18–27.
25. Li Y, Wollnik B, Pabst S, et al. BTNL2 gene variant and sarcoidosis. *Thorax*. 2006;61:273–274.
26. Hedfors E, Lindstrom F. HLA-B8/DR3 in sarcoidosis. Correlation to acute onset disease with arthritis. *Tissue Antigens*. 1983;22:200–203.
27. Ishihara M, Ohno S, Ishida T, et al. Molecular genetic studies of HLA class II alleles in sarcoidosis. *Tissue Antigens*. 1994;43: 238–241.
28. Rutherford RM, Brutsche MH, Kearns M, Bourke M, Stevens F, Gilmartin JJ. HLA-DR2 predicts susceptibility and disease chronicity in Irish sarcoidosis patients. *Sarcoidosis Vasc Diffuse Lung Dis*. 2004;21:191–198.
29. Grunewald J, Brynedal B, Darlington P, et al. Different HLA-DRB1 allele distributions in distinct clinical subgroups of sarcoidosis patients. *Respir Res*. 2010;11:25.
30. Zhou Y, Shen Li, Zhang Y, Jiang D, Li H. Human leukocyte antigen-A, -B, and –DRB1 alleles and sarcoidosis in Chinese Han subjects. *Human Immunol*. 2011;72:571–575.
31. Ina Y, Takada K, Yamamoto M, Morishita M, Senda Y, Torii Y. HLA and sarcoidosis in the Japanese. *Chest*. 1989;95:1257–1261.
32. Todd JA, Acha-Orbea H, Bell JI, et al. A molecular basis for MHC class II-associated autoimmunity. *Science*. 1988;240: 1003–1009.
33. Stern LJ, Brown JH, Jardetzky TS, et al. Crystal structure of the human class II MHC protein HLA-DR1 complexed with an influenza virus peptide. *Nature*. 1994;368:215–221.
34. Zhang L, Chen Y, Wong HS, Zhou S, Mamitsuka H, Zhu S. TEPITOPEpan: extending TEPITOPE for peptide binding prediction covering over 700 HLA-DR molecules. *PLoS One*. 2012;7:e30483.
35. Voorter CE, Drent M, van der Berg-Loonen EM. Severe pulmonary sarcoidosis is strongly associated with the haplotype HLA-DQB1*0602-DRB1*150101. *Human Immunol*. 2005;66:826–835.
36. Bogunia-Kubik K, Tomeczko J, Suchnicki K, Lange A. HLA-DRB1*03, DRB1*11 or DRB1*12 and their respective DR3 specificities in clinical variants of sarcoidosis. *Tissue Antigens*. 2001;57:87–90.
37. Mrazek F, Holla LI, Hutyrova B, et al. Association of tumor necrosis factor-alpha, lymphotoxin-alpha and HLA-DRB1 gene polymorphisms with Löfgre's syndrome in Czech patients with sarcoidosis. *Tissue Antigens*. 2005;65:163–171.
38. Amicosante M, Puxeddu E, Salitini C. Reactivity to mycobacterial antigens by patients with Lofgren's syndrome as a model of phenotypic susceptibility to disease and disease progression. *Am J Respir Crit Care Med*. 2009;179:307–312.
39. Berlin M, Fogdell-Hahn A, Olerup O, Eklund A, Grunewald J. HLA-DR predicts the prognosis in Scandinavian patients with pulmonary sarcoidosis. *Am J Respir Crit Care Med*. 1997;156: 1601–1605.
40. Grunewald J, Eklund A, Olerup O. Human leukocyte antigen class I alleles and the disease course in sarcoidosis patients. *Am J Respir Crit Care Med*. 2004;169:696–702.
41. Sato H, Grutters JC, Pantelidis P, et al. HLA-DQB1*0201: a marker for good prognosis in British and Dutch patients with sarcoidosis. *Am J Respir Cell Mol Biol*. 2002;27:406–412.
42. Stammers M, Rowen L, Rhodes D, Trowsdale J, Beck S. BTL-II: a polymorphic locus with homology to the butyrophilin gene family, located at the border of the major histocompatibility complex class II and class III regions in human and mouse. *Immunogenetics*. 2000;51:373–382.
43. Johnson CM, Traherne JA, Jamieson SE, et al. Analysis of the BTNL2 truncating splice site mutation in tuberculosis, leprosy and Crohn's disease. *Tissue Antigens*. 2007;69:236–241.
44. Tesmer LA, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. *Immunol Rev*. 2008;223:87–113.
45. Facco M, Cabrelle A, Teramo A, et al. Sarcoidosis is a Th1/Th17 multisystem disorder. *Thorax*. 2011;66:144–150.