Report

The *BTNL2* Gene and Sarcoidosis Susceptibility in African Americans and Whites

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The *BTNL2* gene is a member of the B7 receptor family that probably functions as a T-cell costimulatory molecule. It resides in the class II major histocompatibility complex (MHC) region of chromosome 6p and has recently been associated with sarcoidosis susceptibility in a white German population. We sought to replicate the *BTNL2* association in an African American family-based study population ($n = 219$ nuclear families) and two case-control populations—one African American ($n = 295$ pairs) and one white ($n = 366$ pairs). Ten SNPs were detected within a 490-bp region spanning exon/intron 5 of *BTNL2*. Haplotype variation within this region was significantly associated with sarcoidosis in all three study populations but more so in whites ($P = .0006$) than in the African American case-control ($P = .02$) or family-based ($P = .03$) samples. The previously reported *BTNL2* SNP with the strongest sarcoidosis association, *rs2076530,* was also the SNP with the strongest association in our white population ($P < .0001$). The A allele of *rs2076530* results in a premature exon-splice site and increases risk for sarcoidosis (odds ratio = 2.03; 95% confidence interval 1.32–3.12). Although *rs2076530* was not associated with sarcoidosis in either African American sample, a three-locus haplotype that included *rs2076530* was associated with sarcoidosis across all three study samples. Multivariable logistic regression analyses showed that *BTNL2* effects are independent of human leukocyte antigen class II genes in whites but may interact antagonistically in African Americans. Our results underscore the complexity of genetic risk for sarcoidosis emanating from the MHC region.

Sarcoidosis, a multiorgan granulomatous inflammatory disease, probably results from an exaggerated T-cell response to an airborne antigen (American Thoracic Society et al. 1999). Whereas human leukocyte antigen (HLA) genes have long been thought to play a role in sarcoidosis (Martinetti et al. 2002), the high density of immune-related genes and linkage disequilibrium (LD) in the major histocompatibility complex (MHC) region create difficulties in separating out individual gene effects (Cullen et al. 2002; Walsh et al. 2003; Stenzel et al. 2004). Following-up a previously detected HLA linkage to sar-

coidosis (Schurmann et al. 2001), Valentonyte et al. (2005) reported a novel association with *rs2076530,* a coding SNP on exon 5 of the *BTNL2* gene (MIM 606000), that is independent of *HLA-DRB1* sarcoidosis risk alleles. The *rs2076530* G→A transition leads to an alternative splice site that results in an early stop codon and a truncated protein. *BTNL2,* aliases "butyrophilin-like 2" and "BTL-2," is a butyrophilin gene that belongs to the immunoglobulin gene superfamily and is related to the *B7.1* and *B7.2* (*CD80* and *CD86*) costimulatory receptors (Rhodes et al. 2001; Sharpe and Freeman 2002), but its exact function is unknown. Optimal T-cell activation requires antigen engagement of the T-cell receptor with additional costimulatory interactions. CD28, expressed in T cells, binds to either the B7.1 or B7.2 counterreceptors on antigen-presenting cells (Shahinian et al. 1993; Krinzman et al. 1996). Dysfunctional BTNL2 could interfere with normal T-cell regulation (Harding et al. 1992).

The close proximity of *BTNL2* to *HLA-DRB1* and *HLA-DQB1,* which have known sarcoidosis risk alleles

(Sato et al. 2002; Iannuzzi et al. 2003; Rossman et al. 2003), complicates association studies. In populations of African origin, increased haplotype diversity in the MHC class II region may help in differentiating specific gene effects (Just et al. 1997). To determine the consistency of the *BTNL2* gene as a sarcoidosis risk factor across different populations, we characterized variation in the exon/intron 5 region of *BTNL2* in an African American family sample that consisted of 219 nuclear families (686 individuals) and in two case-control samples (295 African American matched pairs and 366 white matched pairs). The study protocols were approved by institutional review boards of all participating centers.

In the African American family sample, which was ascertained primarily from the Henry Ford Health System, diagnosis was confirmed by tissue biopsy in 86% of the index cases and in all index cases with normal (stage 0) chest X-rays. Those subjects without histologic confirmation had radiographic evidence of bilateral hilar adenopathy, a compatible clinical presentation, and were observed for ⩾2 years to verify that no other medical condition could explain the clinical course. Of the 623 eligible probands identified, 359 (58%) were enrolled with one or more first-degree family members. Of those 359 probands, 234 had at least two parents or siblings who donated a blood sample. An additional 10 African American families with sarcoidosis were recruited from outside of the Henry Ford Health System, for a total of 244 families. Both parents were genotyped when available. When one or both parents were unavailable for genotyping, all available full siblings were genotyped. After exclusion of families with Mendelian inconsistencies and insufficient DNA samples for analysis, the final sample used for data analysis comprised 219 nuclear families with 686 individuals (191 parents and 495 siblings), of whom 241 were affected siblings (cases) and 254 were unaffected siblings (controls) (table 1). Detailed descriptions of this study population have been published elsewhere (Rybicki et al. 2003, 2004).

African Americans and whites with sarcoidosis and matched controls who participated in a case-control etiologic study of sarcoidosis (ACCESS) were also genotyped for *BTNL2*. Between November 1996 and June 1999, 736 cases and 706 matched controls entered the study (ACCESS Research Group 1999). Cases met the following inclusion criteria: (1) first tissue confirmation of noncaseating granulomas on biopsy within 6 mo of enrollment, (2) clinical signs or symptoms consistent with sarcoidosis, and (3) aged ⩾18 years. Excluded were individuals with fungal disease or active tuberculosis or who were receiving antituberculosis therapy. Patients with a history of beryllium exposure were excluded unless they had a negative blood beryllium-lymphocyte proliferation test. The clinical characteristics of the study patients were described elsewhere (Baughman et al. 2001).

Table 1

Study Samples

POPULATION CHARACTERISTICS	FINDINGS FOR		
	African American Family Sample	African American Case-Control Sample	White Sample
Cases:			
n	241	295	366
Percentage Male	27.0	26.4	42.6
Mean age ± SD (years)	44.3 ± 9.0	40.3 ± 9.7	43.6 ± 10.6
Controls:			
n	254	295	366
Percentage Male	35.4	26.4	42.6
Mean age ± SD (years)	46.3 ± 10.7	40.3 ± 10.1	43.7 ± 10.5

Controls were recruited by random digit dialing and were matched to cases on the basis of age (within 5 years), sex, and self-reported ethnicity and geographic region. Potential controls who reported a history of sarcoidosis or medical conditions that made the determination of affection status uncertain—for example, granulomatous hepatitis or idiopathic uveitis—were excluded. Of the 706 enrolled case-control pairs, 686 self-reported that they were of white or African American ethnicity. The present study included the 661 pairs in that subset that had sufficient DNA remaining for genotyping (table 1).

Custom primer oligonucleotides for PCR and DNA sequencing were designed from the reference sequence surrounding *rs2076530*, to amplify a 490-bp amplicon from each of the genomic samples. The primers used for amplification and sequencing were 5′-AATGCACAGA-GCATGGAGGTGAG-3′ and 5′-GAAGATACTGGAA-AAGATACAAG-3′. PCR amplification of genomic DNA was performed by standard PCR protocols. Quality control of PCR products was performed by agarose-gel electrophoresis to confirm amplimer size. PCR products were purified using commercial columns and then were sequenced by DNA cycle sequencing by use of BigDye Terminator v3.1 chemistry. Sequence delineation and base calling were performed using automated fluorescent DNA sequencers, Applied Biosystems model 3730xl. For each primer-template combination, a set of two sequencing reactions was performed to get the required fourfold redundancy (2 upstream, and 2 downstream) across the template. SNPs were called for mixed nucleotide positions in which the second peak is ⩾50% of the height of the first peak.

Ten biallelic SNPs were identified in exon/intron 5 of the *BTNL2* gene (table 2). SNPs were detected at nucleotide position (np) 15841 (A/T exon; Asp/Val), np 15843 (C/T exon; Arg/Ter), np 15932 (A/G exon; synonymous *rs2076529*), np 15994 (C/T exon; Ser/Leu), np 16043 (G/A; synonymous *rs9268480*), np 16047 (G/A

Table 2

National Center for Biotechnology Information Accession Numbers, Flanking Sequences, and Frequencies of Exon/Intron 5 *BTNL2* SNPs in African Americans and Whites

The table is available in its entirety in the online edition of *The American Journal of Human Genetics*.

exon; Ala/Thr), np 16071 (A/G exon; premature truncation of mRNA by 4 bp/Gly *rs2076530*), np 16113 (C/T; intron), np 16165 (T/C; intron), and np 16171 (T/C; intron) (dbSNP). Of the 10 identified SNPs, 7 were novel, and 4 of the 7 were coding SNPs that predicted amino acid substitutions at positions 283, 284, 334, and 352 of the BTNL2 protein. For each SNP, the proportion of heterozygous and homozygous genotypes was consistent with Hardy-Weinberg equilibrium in cases and controls across all three populations. Although all 10 SNPs were detected in both African Americans and whites, 4 of the SNPs—*A15841T* (accession number *ss38346932*), *C15843T* (accession number *ss38346933*), *G16047A* (accession number *ss38346937*), and *T16171C* (accession number *ss38346941*)—were rare (frequency <1%) in whites. Of the remaining six SNPs, four (*G16043A*, *A16071G*, *C16113T* [accession number *ss38346939*], and *T16165C* [accession number *ss38346940*]) captured >95% of the haplotype variation within this region. Since *A16071G* was in complete LD with *A15932G*, is known to be a functional SNP, and was the SNP reported elsewhere to be associated with sarcoidosis (Valentonyte et al. 2005), we chose it over *A15932G* for building haplotypes across the region.

To determine whether an allele at the locus of interest was associated with sarcoidosis, we used a family-based association test statistic, *S*, calculated using the family-based association testing software FBAT (Laird et al. 2000). *BTNL2* haplotype data were analyzed using the haplotype (HBAT) module in the FBAT software (Horvath et al. 2004). Similar to FBAT, HBAT provides a test in a family-based study that is efficient and robust to population admixture, phenotype distribution specification, and ascertainment based on phenotypes. As with FBAT, HBAT can also handle missing parental genotypes and/or missing phase in both offspring and parents. HBAT yields either haplotype-specific (univariate) tests or multihaplotype (global) tests. To increase statistical power across the two African American samples, we pooled *P* values for the haplotype tests. The overall *P* value was computed by summing the natural logs of each *P* value, multiplying by −2, and evaluating the result on a χ^2 distribution curve with 4 df (2 × the number of tests).

Case-control data were analyzed using matched logistic regression methods with the PHREG procedure in SAS (SAS Institute). Haplotype data were analyzed with CHAPLIN (case-control haplotype inference) software, which uses a retrospective likelihood method that sums over all possible pairs of haplotypes that are consistent with the genotype data (Epstein and Satten 2003). LD between selected pairs of loci in the case-control samples was estimated in two stages. First, since haplotypes were not directly observable, maximum-likelihood haplotype frequencies were computed using the ARLEQUIN genetic analysis software (Excoffier and Slatkin 1995). These haplotype frequencies were then used to calculate the normalized measure for LD between loci, D' (Lewontin 1964; Hedrick 1987).

Four haplotype-tagging SNPs in exon/intron 5 of *BTNL2* form five haplotypes with variable frequencies and associations with sarcoidosis in the three different study populations (table 3). The most frequent haplotype, G-A-C-T (haplotype 1), conferred 1.5-fold increased risk for sarcoidosis ($P = .00004$) in whites. Haplotype 1 was observed in 66% of white cases but only 55% of controls. In the white sample, the A allele at np 16071 had an odds ratio (OR) for heterozygotes (AG vs. GG) of 1.70 (95% CI 1.08–2.67) and an OR for homozygotes (AA vs. GG) of 2.63 (95% CI 1.64–4.24). This result is similar to what was observed in the original report of *BTNL2* (Valentonyte et al. 2005) in a white German population (OR for AG vs. GG of 1.60; OR for AA vs. GG of 2.75). In our white sample, individuals who had one or more copies of the A allele had a twofold increased risk of sarcoidosis (OR = 2.03; 95% CI 1.32–3.12); the population attributable risk for AG heterozygotes and AA homozygotes was 27.5%. Haplotype 1 was not associated with sarcoidosis in either African American sample. The next most frequent haplotype in African Americans, G-G-T-T (haplotype 2), was not associated with sarcoidosis in either the family or case-control sample. In whites, haplotype 2 was underrepresented in cases (OR = 0.60; $P = .006$). The second most frequent haplotype in the white population—haplotype 3, A-G-C-T—was also underrepresented in whites with sarcoidosis (OR = 0.74; $P = .02$). A similar effect was observed in African American families (transmission distortion [TD] = 0.76; $P = .02$) but not in the African American case-control sample. Haplotype 4, A-G-C-C, was underrepresented in cases across all three samples, but only in the African American case-control sample did this haplotype show a significant negative association with sarcoidosis (OR = 0.44; $P = .001$). The least frequent of the five haplotypes, G-G-C-T (haplotype 5), conferred a modest increased risk of sarcoidosis in the African American family sample (TD = 1.53; $P = .04$) but showed no association with sarcoidosis in the other two samples. Since the first three bases of haplotypes 3 and 4 were the same—A-G-C—we examined the risk associated with this haplotype across all three samples.

The A-G-C haplotype was found in cases significantly less often than expected in all three samples: TD = 0.80 (*P* = .01) in the African American family sample; OR = 0.65 (*P* = .009) in the African American case-control sample; and OR = 0.73 (*P* = .007) in the white sample.

The haplotype distribution across *BTNL2* exon/intron 5 was significantly associated with sarcoidosis in all three populations. This association in African Americans was more modest, *P* = .03 in the family sample and *P* = .02 in the case-control sample, compared with the white sample (*P* = .0006). Pooling the independent *P* values from the two African American samples resulted in an overall haplotype association *P* value of .006. Other less common variants in this region specific to African Americans were increased in subjects with sarcoidosis in the African American families, most notably *A15841T* (TD = 1.50; *P* = .06), *C15843T* (TD = 1.61; *P* = .03), *C15994T* (accession number *ss38346935*) (TD = 1.66; *P* = .01), and *T16171C* (TD = 1.70; *P* = .01). These four variants were in strong LD and had an increased transmission to affected offspring in nuclear families (TD = 1.70; *P* = .01). Of these four variants, three (*A15841T, C15843T,* and *C15994T*) coded for predicted amino acid changes, and an additional fourth novel non-synonymous variant specific to African Americans was found at np 16047. To determine whether these potential functional coding changes add anything to the haplotypic variation analysis depicted in table 3, we reanalyzed the haplotypes with these four nonsynonymous SNPs. In the family sample, all transmissions of the four-locus variant haplotype of the nonsynonymous SNPs occurred in tandem with haplotype 5 and therefore did not add any additional haplotype-variation information. In the African American case-control sample, the four-locus variant haplotype occurred separate from the other five haplotypes listed in table 3 but was observed with
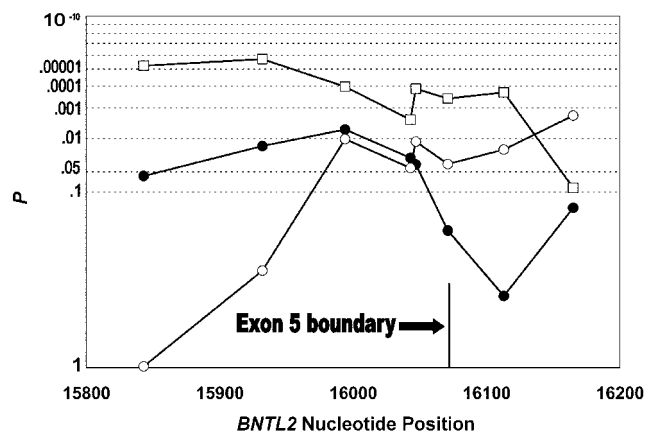

Figure 1   Level of association between sarcoidosis and variation in the exon/intron 5 region of *BTNL2* on the basis of the three-SNP haplotype window moving across the region. The three study samples analyzed include 219 African American nuclear families (*blackened circles*), 295 African American case-control pairs (*unblackened circles*), and 366 white case-control pairs (*unblackened squares*).

an equal frequency in cases and controls (four times in each); therefore, inclusion of these additional variants in the haplotype calculation had only a nominal effect on the statistical test for case-control differences in *BNTL2* haplotype variation. In separate testing of three-locus haplotype windows moving across this region, the lowest *P* value of association in the white population was centered at *A15932G,* but highly statistically significant associations were observed for haplotypes across most of the exon/intron 5 region (fig. 1). In the two African American samples, the lowest *P* values were observed at the far end of the exon 5 boundary. When we extended the haplotype window outside of this boundary, the level of association increased in the African American case-

Table 3

Association between *BTNL2* Haplotypes and Sarcoidosis in African Americans and Whites

	FINDINGS FOR								
	African American Family Sample (*n* = 241 cases; 254 controls)			African American Case-Control Sample (*n* = 295 pairs)			White Sample (*n* = 366 pairs)		
HAPLOTYPE[a]	Frequency[b]	TD	*P*	Frequency[b]	OR	*P*	Frequency[b]	OR	*P*
G-A-C-T	72.4	1.04	.26	68.9	1.16	.23	60.3	1.56	.00004
G-G-T-T	10.7	1.01	.89	13.4	1.26	.17	9.2	.60	.006
A-G-C-T	8.1	.76	.02	8.9	.92	.68	23.2	.74	.02
A-G-C-C	6.4	.86	.37	6.6	.44	.001	5.9	.83	.39
G-G-C-T	2.3	1.53	.04	2.2	.85	.69	1.3	1.04	.93

[a] Nucleotide sequence at *BTNL2* np 16043 (*rs9268480*), np 16071 (*rs2076530*), np 16113, and np 16165.

[b] Haplotype frequency in overall sample.

control sample but decreased in the African American family sample.

To determine which SNP or set of SNPs most influenced the observed haplotype associations with sarcoidosis, we performed a stepwise model-building procedure for each sample. In the African American family sample, a model with the *G16043A* SNP (*rs2076529*) represented the best-fitting parsimonious model ($P = .04$). In the African American case-control sample, a similar result was observed, in that a model with only one SNP, *T16165C,* represented the best-fitting parsimonious model ($P = .02$). In the white sample, the best-fitting model included two SNPs, *G16043A* and *C16113T* ($P = .00004$).

Because of the proximity of *BTNL2* to HLA class II genes, we next investigated the extent of LD for the four *BTNL2* haplotype-tagging SNPs and the HLA class II genes *DRB1, DQB1,* and *DPB1* (table 4). Of the four *BTNL2* SNPs, *DRB1* had the highest D' values and *DRB3* had the lowest. In general, LD between the HLA class II loci and *BTNL2* was stronger in African Americans than in whites. An examination of allele-specific LD between *HLA-DRB1* and the four *BTNL2* SNPs showed that, although most allele pairs were in complete LD ($D' = 1$), many allele pairs had D' values <1 (fig. 2*A* and 2*B*). In both whites and African Americans, the *BTNL2* SNPs *G16043A* and *A16071G* had the most allele pairs in less-than-complete LD, whereas *T16165C* had the fewest. Because of the strong LD between many of the *HLA-DRB1* alleles and *BTNL2* and the previously found associations between *HLA-DQB1* and *HLA-DRB1* in these samples (Iannuzzi et al. 2003; Rossman et al. 2003), we performed multivariable logistic regression to determine the potential for confounding and effect modification of *BTNL2* allelic effects from HLA class II risk alleles. In the two case-control samples for which data for both *BTNL2* and HLA class II were available, we tested three different types of logistic regression models: (1) models with a term for only the *BTNL2* variant to estimate the crude OR for this variant in the analysis subsample, (2) models with terms for the *BTNL2* variant and HLA class II risk allele to estimate effects of confounding, and (3) models with terms for the *BTNL2* variant, the HLA class II risk allele, and a cross-product interaction term to estimate effect modification.

The *BTNL2* alleles tested were those found significantly associated with sarcoidosis in the two case-control samples. The tested HLA class II alleles represent the alleles that had the strongest race-specific associations with sarcoidosis that were reported elsewhere for these samples (Rossman et al. 2003). For African Americans, we examined HLA class II allele effects on the association between the variant alleles of *BTNL2* exon 5 SNPs *G16043A* and *T16165C* (table 5). In general, the

Table 4

LD between HLA Class II Genes and Exon/Intron 5 *BTNL2* Haplotype-Tagging SNPs in Cases and Matched Controls

BTNL2 VARIANT AND HLA CLASS II LOCUS	D' FOR	
	African Americans ($n = 187$ pairs)	Whites ($n = 260$ pairs)
G16043A (*rs9268480*):		
HLA-DRB1	.800	.835
HLA-DRB3	.137	.073
HLA-DQB1	.698	.658
HLA-DPB1	.209	.310
A16071G (*rs2076530*):		
HLA-DRB1	.794	.607
HLA-DRB3	.100	.175
HLA-DQB1	.634	.485
HLA-DPB1	.209	.211
C16113T:		
HLA-DRB1	.860	.615
HLA-DRB3	.211	.333
HLA-DQB1	.713	.480
HLA-DPB1	.251	.234
T16165C:		
HLA-DRB1	.947	.860
HLA-DRB3	.166	.146
HLA-DQB1	.893	.693
HLA-DPB1	.537	.349

four HLA class II alleles associated with sarcoidosis in this sample did not confound the relationship between *G16043A* or *T16165C* and sarcoidosis. There was some suggestion of negative interaction with *DRB1*1201* and positive interaction with *DPB1*1101,* but these interaction ORs did not reach statistical significance. In an analysis that combined all four HLA class II risk alleles into one risk group, we observed a statistically significant ($P = .01$) negative interaction between the *BTNL2* SNP *G16043A* and the HLA class II risk alleles. A similar albeit weaker negative interaction was observed between the *BTNL2* SNP *G16043A* and the HLA class II risk alleles in African Americans.

In our sample of whites, we examined HLA class II allele effects on associations between the variant allele of three *BTNL2* exon 5 SNPs: *G16043A, A16071G,* and *C16113T.* For *G16043A* and *A16071G,* similar nominal confounding effects were observed for the four HLA class II alleles associated with sarcoidosis. *HLA-DRB1*0401* and *HLA-DRB1*1501* slightly attenuated the OR of the *BTNL2* risk allele, whereas *DRB1*0402* slightly increased the OR. A parameter for the combined HLA class II risk alleles slightly attenuated the OR for risk alleles of *G16043A* and *A16071G.* For *C16113T,* an adjustment for the combined effect of HLA class II risk alleles decreased the OR from 0.66 to 0.58. Several interaction ORs for joint *BTNL2*-HLA class II allelic effects >2 or <0.5 were observed, but none approached statistical significance.
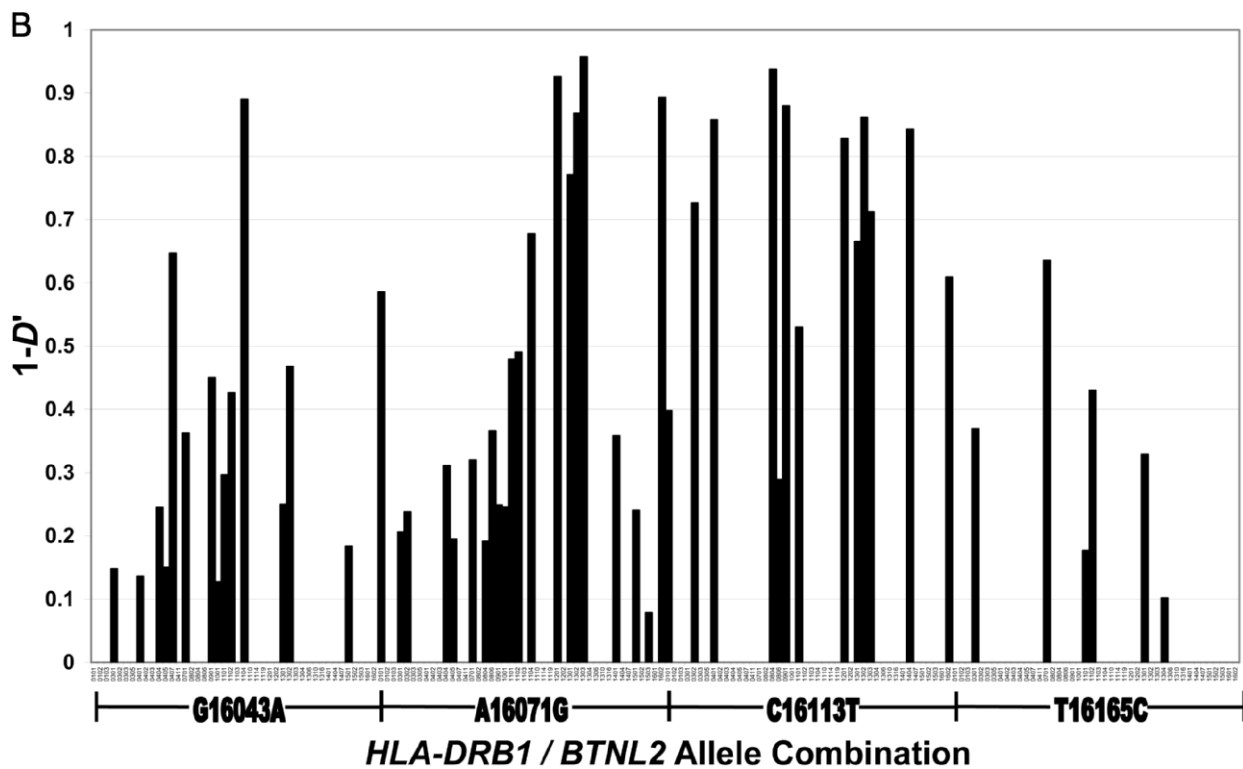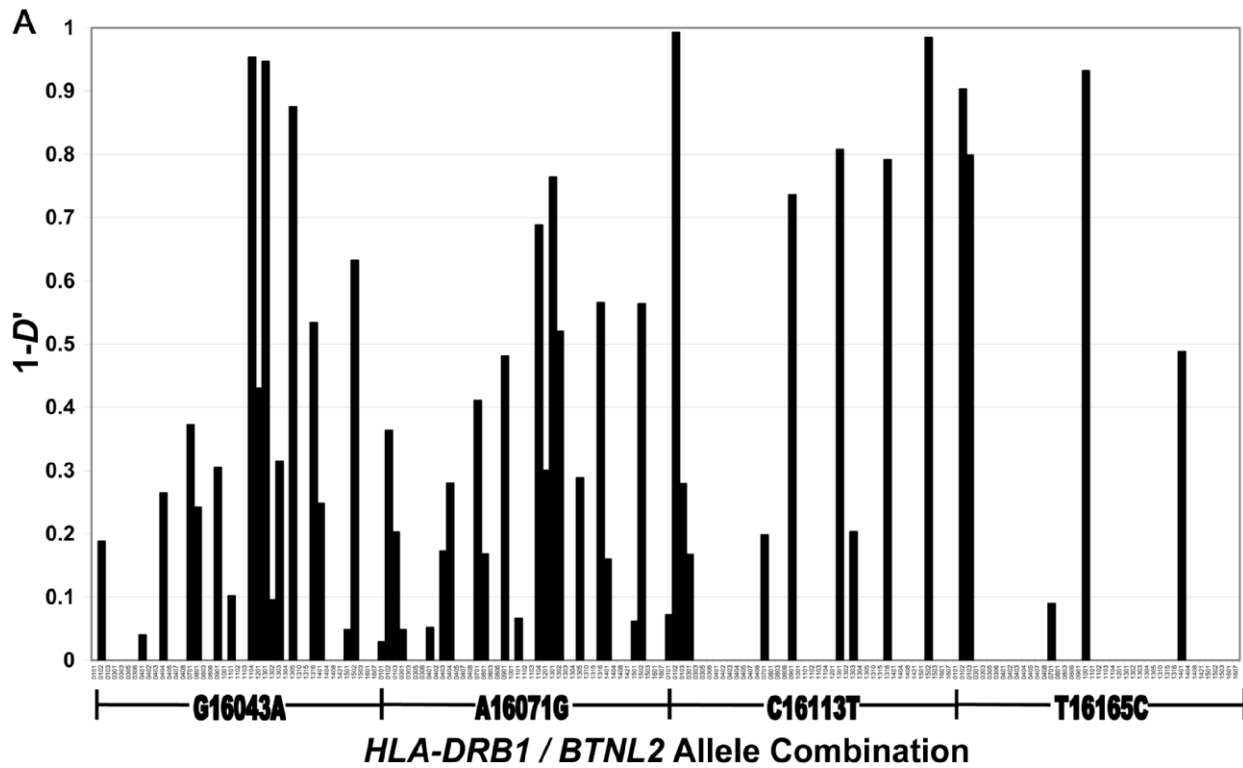
Figure 2   *A,* Incomplete disequilibrium (as measured by $1 - D'$) between allele pairs of *HLA-DRB1* and the four *BTNL2* exon/intron 5 haplotype-tagging SNPs (*G16043A* [*rs9268480*], *A16071G* [*rs2076530*], *G16113T,* and *T16165C*) in whites with sarcoidosis and matched controls (*n* = 260 pairs). *B,* Incomplete disequilibrium (as measured by $1 - D'$) between allele pairs of *HLA-DRB1* and the four *BTNL2* exon/ intron 5 haplotype-tagging SNPs (*G16043A* [*rs9268480*], *A16071G* [*rs2076530*], *G16113T,* and *T16165C*) in African Americans with sarcoidosis and matched controls (*n* = 187 pairs).

Table 5

HLA Class II Confounding and Effect Modification of Associations between Sarcoidosis and Variant Alleles of *BTNL2* Exon/Intron 5 SNPs in Case-Control Pairs

Race, SNP, and Class II Allele	aOR (*P*)	iOR (*P*)
African American (*n* = 187 pairs):		
G16043A (*rs9268480*):		
No allele	1.62 (.03)	...
*DRB1*1101*	1.63 (.03)	.67 (.42)
*DRB1*1201*	1.58 (.04)	.26 (.13)
*DRB1*1503*	1.74 (.01)	.72 (.64)
*DPB1*1101*	1.49 (.08)	1.52 (.33)
All four class II risk alleles	1.61 (.03)	.32 (.01)
T16165C:		
No allele	2.10 (.04)	...
*DRB1*1101*	2.10 (.04)	1.05 (.94)
*DRB1*1201*	2.14 (.04)	.38 (.46)
*DRB1*1503*[a]	2.27 (.02)	
*DPB1*1101*	1.92 (.07)	1.47 (.52)
All four class II risk alleles	2.06 (.05)	.45 (.21)
White (*n* = 260 pairs):		
G16043A (*rs9268480*):		
No allele	1.33 (.04)	...
*DRB1*0401*	1.14 (.37)	1.07 (.90)
*DRB1*0402*	1.51 (.005)	.39 (.44)
*DRB1*1101*	1.29 (.08)	.50 (.20)
*DRB1*1501*	1.16 (.31)	.61 (.21)
All four class II risk alleles	1.35 (.03)	.85 (.57)
A16071G (*rs2076530*):		
No allele	1.48 (.003)	...
*DRB1*0401*	1.36 (.02)	.80 (.65)
*DRB1*0402*	1.61 (.0005)	.36 (.41)
*DRB1*1101*	1.42 (.008)	.92 (.88)
*DRB1*1501*	1.29 (.06)	.79 (.53)
All four class II risk alleles	1.40 (.01)	1.05 (.86)
C16113T:		
No allele	1.51 (.05)	...
*DRB1*0401*	1.72 (.01)	.87 (.91)
*DRB1*0402*[a]	1.46 (.08)	
*DRB1*1101*	1.45 (.08)	3.09 (.23)
*DRB1*1501*	1.44 (.10)	1.35 (.59)
All four class II risk alleles	1.28 (.26)	1.31 (.57)

NOTE.—aOR = adjusted OR for the variant allele; iOR = interaction OR.

[a] Parameter not estimable for iOR and related *P* value.

On the basis of our findings in two different African American samples, it appears that significant allelic heterogeneity exists at the *BTNL2* locus. Resulting functional differences in *BTNL2* may be attenuated in the sarcoidosis phenotype because of the high redundancy of costimulatory molecules in the human immune system. Whereas greater allelic diversity may explain in part the more modest *BTNL2* association observed in African Americans, an alternative explanation may be an antagonistic effect of HLA class II risk alleles on *BTNL2*-associated risk. The gene-gene interaction results reported by Valentonyte et al. (2005), although not conclusive, suggest that *HLA-DRB1* is a risk factor for sarcoidosis only in the presence of the truncating *BTNL2*

allele. Our interaction analyses of HLA class II risk alleles and *BTNL2* in African Americans suggest that the risk-bearing alleles of these two loci negatively interact. The greater age and selective pressure on immune-related genes in populations of African descent (Lazarus et al. 2002; Cao et al. 2004) (i.e., the explanation for the greater genetic diversity observed in African Americans) could explain this putative canceling effect of HLA class II and *BTNL2,* with regard to sarcoidosis risk in African Americans.

The exact functional role of *BTNL2* in the costimulatory system remains to be elucidated. In demonstrating an association between *BTNL2* in both white Americans and African Americans, we confirmed the initial report of the *BTNL2* gene association with sarcoidosis in white Germans (Valentonyte et al. 2005). We also demonstrated that *BTNL2* risk effects observed in the white samples were independent of *HLA-DRB1* associations found elsewhere for these samples (Rossman et al. 2003), but we found suggestive evidence for negative interactions between *BTNL2* and HLA class II in African Americans. Larger samples will be needed to better define these effects and to estimate them with a higher degree of statistical certainty (Garcia-Closas and Lubin 1999). Future studies should focus on defining the immunological role of *BTNL2* in granuloma formation in relation to HLA class II.

Acknowledgments

Web Resources

Accession numbers and URLs for data presented herein are as follows:

dbSNP, http://www.ncbi.nlm.nih.gov/SNP/ (for *A15841T* [accession number *ss38346932*], *C15843T* [accession number *ss38346933*], *A15932G* [accession number *rs2076529*], *C15994T* [accession number *ss38346935*], *G16043A* [accession number *rs9268480*], *G16047A* [accession number *ss38346937*], *A16071G* [accession number *rs2076530*], *C16113T* [accession number *ss38346939*], *T16165C* [accession number *ss38346940*], and *T16171C* [accession number *ss38346941*])

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim/ (for *BTNL2*)

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