Published in final edited form as:
Genes Immun. 2013 January ; 14(1): 13–18. doi:10.1038/gene.2012.48.

Association of *ANXA11* genetic variation with sarcoidosis in African Americans and European Americans

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Abstract

A recent genome-wide association study in a German population and two subsequent studies in European populations found that a non-synonymous single-nucleotide polymorphism (SNP), rs1049550, within the *annexin A11* (*ANXA11*) gene was associated with susceptibility to sarcoidosis. We sought to identify additional *ANXA11* variants independently associated with sarcoidosis, determine whether any sarcoidosis-associated *ANXA11* variants were associated with chest radiographic phenotypes, and explore human leukocyte antigen (*HLA*) SNP–SNP interactions with *ANXA11*. A total of 209 SNPs spanning 100 kb including the 5' promoter, coding, and 3' untranslated regions of *ANXA11* were genotyped for 1689 sarcoidosis cases and 1252 controls. After adjustment for rs1049550, two additional novel *ANXA11* sarcoidosis associations were identified only in African Americans—rs61860052 (odds ratio (OR) = 0.62; 95% confidence interval (CI) = 0.40–0.97) and rs4377299 (OR = 1.31; 95% CI = 1.06–1.63). These associations were more pronounced in radiologically-classified Scadding stage IV sarcoidosis cases. We also identified a significant SNP–SNP interaction between rs1049550 and a sarcoidosis risk SNP (rs9268839) near the *HLA–DRA* locus. This further genetic dissection of *ANXA11* may provide additional insight into the immune dysregulation characteristic of sarcoidosis pathophysiology.

Keywords

annexins; lung; African Americans; polymorphism; HLA class II genes

INTRODUCTION

Sarcoidosis results from a dysregulated immune response to one or more yet-unidentified antigens,[1] leading to infiltration of monocytes, macrophages, and activated T-lymphocytes, with subsequent formation of non-caseating granulomas. Pulmonary involvement ranges

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

Supplementary Information accompanies the paper on Genes and Immunity website (http://www.nature.com/gene)

from spontaneous resolution with no impairment to fibrotic lung disease with respiratory failure. Patterns of family clustering and differences in racial incidence suggest that genetic factors contribute to disease risk and severity.[1,2]

The human leukocyte antigen (HLA) region of chromosome 6 has been consistently associated with sarcoidosis risk. The class I HLA-B8 antigen was the first to be associated with sarcoidosis.[3] Subsequent studies with large sample sizes have found associations between the HLA class II molecules DRB1 and DQB1, and sarcoidosis risk.[4,5] As exposure to environmental agents likely initiates the disease process, susceptibility to sarcoidosis may be influenced by the ability of HLA class II molecules to present antigens for immune recognition.[4]

In a genome-wide association study (GWAS) of 989 German individuals, Hofmann *et al.*[6] identified and validated a sarcoidosis susceptibility variant, rs1049550 (R230C), a non-synonymous single-nucleotide polymorphism (SNP) residing in the first of four annexin core domains within the gene annexin A11 (*ANXA11*). The sarcoidosis association with the *ANXA11* rs1049550 SNP has since been independently confirmed by case–control studies in two different European populations.[7,8] In our recent GWAS study of sarcoidosis, we also found that rs1049550 was associated with sarcoidosis in African Americans, but unlike the original German study,[6] this association did not reach genome-wide significance.[9]

Annexins are a family of calcium-dependent, phospholipids-binding proteins involved in cell division and apoptosis[10] and linked to autoimmune and other chronic disorders.[11] ANXA11 also has an essential role in the terminal phase of cell cytokinesis.[12] ANXA11 and the HLA class II molecules DRB1, DRB2 and DRA are among the few proteins that are detectable in human B-cell exosomes.[13] Exosomes are thought to induce immune responses or tolerance depending on their cellular origin,[14] and their role in the activation of B cells in sarcoidosis has been proposed.[15] Such activation could lower the threshold for T-cell activation. Given this potential common pathogenic pathway in sarcoidosis and physical proximity in exosomes, it is possible that the established genetic associations between both *ANXA11* and HLA class II genes and sarcoidosis risk depend on one another and reflect a more complex interaction, which has not been explored to date.

In the United States, the incidence and severity of sarcoidosis vary by ethnicity—sarcoidosis is approximately three times more common (35.5/100 000 versus 10.9/100 000) and more likely to be chronic and fatal among African Americans than European Americans.[16,17] We have previously used stratified linkage analysis to demonstrate that genetic influences on sarcoidosis risk vary by ancestry[18] and more recently shown evidence for ancestry-specific disease loci.[19] Using a population comprised of a large case–control and family sample of African Americans and a smaller case–control sample of European Americans, we sought to identify additional independent variants within the *ANXA11* gene region that influence risk of disease development and severity, and to evaluate whether variation in the *HLA* region modified the sarcoidosis risk associated with *ANXA11*.

RESULTS

Confirmation of association with SNP rs1049550 and sarcoidosis in US sample

In total, 25 SNPs in and around *ANXA11* were significantly associated with sarcoidosis risk at the $P<0.05$ level (Table 1) in either African Americans or European Americans. Among African Americans, the most significant allelic association was the minor C allele at rs1049550 ($P = 0.001$), previously identified by Hofmann *et al.*[6] In European Americans, the minor C allele frequency was similarly significantly higher in controls than cases (0.42 versus 0.36, $P = 0.012$), with a decreased risk of sarcoidosis on the additive scale (odds ratio

(OR) = 0.77; 95% confidence interval (CI) = 0.63–0.95; $P = 0.011$). Similarly, in African Americans, the frequency of the minor allele was increased in controls (0.17 versus 0.15) with a decreased risk on the additive scale (OR = 0.84; 95% CI = 0.72–0.96; $P = 0.029$). Although the risk estimate is slightly stronger in European Americans, overlap in CIs suggests that the additive effect of this allele is similar in both groups. Moreover, when the primary analytical results were combined across African Americans and European American samples via meta-analysis, the association of rs1049550 ($P = 5.55*10^{-5}$) was significant after Bonferroni correction. Three additional SNPs met the Bonferroni correction threshold: rs2573375 ($P = 3.23*10^{-5}$), rs1079242 ($P = 7.52*10^{-5}$) and rs1953600 ($P = 1.42*10^{-4}$); notably, each of these SNPs was in linkage disequilibrium with rs1049550 (Table 1). The meta-analysis results for the 25 SNPs in Table 1 are presented in Supplementary Table 1.

Additional SNPs in ANXA11 associated with risk of sarcoidosis

As several *ANXA11* SNPs associated with sarcoidosis risk were also in linkage disequilibrium with rs1049550 (Table 1), we performed a forward-stepwise regression analysis of those SNPs univariately associated with risk. This conditional analysis did not reveal any additional SNPs that were significantly associated with sarcoidosis at the 0.05 level in European Americans. However, two additional *ANXA11* variants were identified in African Americans.

The multivariable *ANXA11* SNP model for sarcoidosis risk in African Americans is presented in Table 2. After conditioning on rs1049550, the most significantly associated SNP was a variant originally identified from the 1000 Genomes Project catalog, located at position 81 961 711 (rs61860052) within the first intron of *ANXA11.* The minor A allele was significantly associated with decreased risk of sarcoidosis before and after adjusting for rs1049550. The OR associated with each minor allele remained statistically significant after accounting for other SNPs. The second additional significant SNP (rs4399277) in this analysis was located in an intergenic region upstream of the *PLAC9* gene, centromeric to *ANXA11* at position 81 881 022. Carriers of the minor A allele had a significantly increased risk of sarcoidosis; the OR associated with each minor allele was 1.31 (95% CI = 1.06–1.63) after accounting for other SNPs.

Among European Americans, no additional SNPs were significant at the 0.05 level after conditioning on rs1049550. However, the analysis of SNP rs6180052 (after accounting for rs1049550) resulted in a *P*-value of 0.09. Given this suggestive result in European Americans, we attempted to validate the association among a separate sample of 711 sarcoidosis cases and 1083 controls from Germany. However, after accounting for rs1049550, rs61860052 was not associated with risk of sarcoidosis ($P = 0.72$).

ANXA11 SNPs and associations with radiographic phenotypes

To investigate whether variants in the *ANXA11* region were associated with radiographic phenotype, we analyzed the relationship of *ANXA11* variants to Scadding stage (in comparison with controls) after a minimum of 2 years follow-up in the subset of our African-American sample for which radiographic staging data were available (Table 3).

For SNPs rs1049550 and rs61860052, the association of the minor alleles was stronger in cases with persistent disease than those with resolved disease; however, there was no evidence for statistically significant heterogeneity between these associations. For SNP rs4399277, the strongest association was found in cases with Scadding stage IV disease, where the A allele was associated with a 1.52-fold increase in the odds of stage IV disease on an additive scale.

Variation across HLA moderates the effect of rs1049550

The *HLA* region contains multiple genetic loci associated with risk of sarcoidosis.[20] To determine whether variation within these loci modifies sarcoidosis risk associated with *ANXA11* rs1049550, we carried out tests of multiplicative interaction using *HLA* SNP data from our GWAS of sarcoidosis risk.[9] To limit the number of tests, only *HLA* SNPs with a minor allele frequency >0.1 and a marginal SNP association (P<0.1) were selected; this resulted in the evaluation of 2029 European American and 1779 African American SNP–SNP interaction models, which corresponded to 3305 unique SNPs and 503 evaluated in both ethnic groups. The interaction OR (with 95% CIs) and P-values are presented for each ethnicity and combined via meta-analysis in Supplementary Table 2 for those interactions with P-values < 0.05 in either ethnicity or in the combined group.

There were no race-specific interactions that were statistically significant after Bonferroni correction for multiple tests. The most significant SNP interaction with *ANXA11* rs1049550 for African Americans was with SNP rs9268839 ($P = 0.004$), and the most significant interaction for European Americans was with SNP rs13211921 ($P = 3.43*10^{-4}$); both of these SNPs are located near the *HLA–DRA* gene. When the evidence for interaction was combined for African Americans and European Americans via meta-analysis, rs9268839 was the only SNP that demonstrated a significant cross-ethnic association ($P = 1.46*10^{-5}$) after Bonferroni correction. Of note, the G allele of *HLA* SNP rs9268839 was univariately associated with a decreased risk of sarcoidosis in both European Americans (OR = 0.67; 95% CI = 0.54–0.84; $P = 3.7*10^{-4}$) and African Americans (OR = 0.71; 95% CI = 0.61–0.83; $P = 9.8*10^{-6}$).

In Table 4, the interaction is shown by the genotypic ORs for rs1049550 (relative to homozygous TT carriers) stratified by rs9268839 genotype for both African Americans and European Americans. If no interaction exists, one would expect to see a similar protective effect of the C allele for rs1049550 across each genotype for rs9268839. In contrast, the results in Table 4 show that the protective effect of the C allele of rs1049550 is limited to those who carry the AA genotype at rs9268839 in both ethnicities. Further, individuals homozygous for the G allele at rs9268839 appear to have an increased risk of sarcoidosis associated with the C allele of rs1049550. Among homozygous carriers of the G allele at rs9268839, the increased risk found for the rs1049550 C allele attained a suggestive level of significance in African Americans (OR = 1.94; 95% CI = 0.92–3.18; $P = 0.09$) with a more modestly elevated OR also observed in European Americans (OR = 1.25; 95% CI = 0.69–2.25; $P = 0.46$); the lack of a statistically significant effect in this stratified analysis may be due to the small number of homozygous G allele carriers at rs9268839 in the sample (African Americans: 38 cases, 52 controls; European Americans: 44 cases, 56 controls). Overall, these results suggest that the effect of *ANXA11* rs1049550 is dependent on the genotype of *HLA* rs9268839.

DISCUSSION

This is the first study to thoroughly characterize genetic variation in *ANXA11* beyond that captured by the earliest GWAS platforms. Hoffman *et al.*[6] previously reported an association between the C allele of the non-synonymous SNP rs1049550 and significantly decreased risk of sarcoidosis in a German population.[6] This association was replicated in two more European populations by Li *et al.*[7] and Mrazek *et al.*[8] As part of the first sarcoidosis GWAS done in African Americans, we further confirmed this association in a US population that includes a large sample of African Americans.[9] In this study, we have also demonstrated that rs1049550 is significant cross-ethnically at the gene level after adjustment for the single SNP association tests performed.

In this study, we identified additional novel independent loci associated with sarcoidosis risk in *ANXA11*. The minor A allele of SNP rs61860052 was associated with a protective effect on sarcoidosis in African Americans. This SNP is newly cataloged by the 1000 Genomes Project with a minor allele frequency of 2% in African Americans and 11% in European Americans. Notably, this variant falls within a region of intron 1 of *ANXA11* containing promoter and enhancer elements as cataloged by the Encyclopedia of DNA Elements (ENCODE) project (genome.ucsc.edu/ENCODE). Although its location may suggest a possible functional role for this SNP in *ANXA11* transcription, it should be noted that this finding did not retain significance after Bonferroni correction for multiple tests in ethnicity-specific or combined analyses in our study.

Previous studies of the association between *ANXA11* and sarcoidosis severity showed mixed results. Although Li *et al.*[7] found no association between acute and chronic forms of sarcoidosis and allelic frequencies of SNPs rs1049550 and rs2573346, Mrazek *et al.*[8] found that the rs1049550 C allele was less frequent in patients with infiltration of lung parenchyma compared with those with isolated hilar lymphadenopathy. For the African American sample only, our study examined *ANXA11* variants in relation to Scadding stage after a minimum of 2 years of follow-up. Although rs1049550 showed no differential association with Scadding stage, the minor alleles of the novel risk loci identified in our analysis—rs61860052 and rs4399277—were more strongly associated with radiographically persistent disease (stage IV), although the *P*-value for rs61860052 ($P = 0.11$) did not fall below 0.05.

We also identified a significant SNP–SNP interaction between the *ANXA11* SNP rs1049550 and the *HLA* SNP rs9268839, which withstood a Bonferonni correction for multiple testing in the full sample, but not when stratified by ethnicity. Despite its location in an intergenic region with unknown function, rs9268839 is in linkage disequilibrium with variants associated with lung function among individuals with cystic fibrosis (SNP rs9268905)[21] as well as the autoimmune diseases rheumatoid arthritis (SNP rs9268853)[22] and ulcerative colitis (SNPs rs9268853 and rs2395185).[23–26] Although this interaction requires validation in additional studies, we believe this is the first SNP–SNP interaction to be identified as potentially influencing sarcoidosis risk.

The observed statistical interaction between *ANXA11* and *HLA* genotypes has biologic plausibility. Sarcoidosis is commonly characterized as a dysregulated immune response to an environmental antigen.[27–30] If a HLA class II molecule cannot recognize and/or present putative sarcoidosis antigens to the immune system, then the immune cascade characteristic of sarcoidosis pathogenesis will not be initiated—thereby eliminating the influence of *ANXA11* variation on inflammatory cell proliferation and apoptosis. One possible mechanism of a biological interaction between these genes may be mediation of cellular immunity via exosomes; both ANXA11 and several HLA molecules are among a limited number of proteins found within exosomes produced by B cells, and ANXA11 co-precipitates with major histocompatibility complex class II proteins.[13] In addition, higher levels of exosomes expressing major histocompatibility complex class II proteins have been detected in bronchoalveolar lavage fluid from sarcoidosis patients,[15] raising the possibility that exosomes have a role in the amplification of the T-cell response observed in sarcoidosis.[31]

In conclusion, through extensive characterization of genetic variation in and around *ANXA11*, we have found suggestive evidence that additional, less-common variants are independently associated with risk of disease susceptibility and radiographic stage, which suggests that the relationship between genetic variation in *ANXA11* and sarcoidosis risk is more complex among people of African ancestry. In addition, we demonstrate that the association between *ANXA11* rs1049550 may depend on variation near *HLA–DRA*, a gene

coding for a class II molecule expressed in antigen-presenting cells that may have a role in sarcoidosis pathogenesis. In sum, these findings emphasize the necessity of exploring the full range of variation in GWAS-identified regions in a multi-ethic manner, and highlight the importance of moving beyond single gene models to explore gene–gene interactions that may provide insight into the genetic architecture of sarcoidosis.

MATERIALS AND METHODS

Study sample and case phenotyping

DNA samples and subject data were collected from three previously conducted studies: a multi-site case-control study (ACCESS);[32] a multi-site affected sibling-pair study (SAGA);[33] and a family-based study recruited through the Henry Ford Health System (HFHS).[5] The ACCESS sample included a total of 1266 subjects (466 African Americans and 800 European Americans). The SAGA sample included 730 African American subjects, and the HFHS sample included 945 subjects. Among the African American subjects, 595 were familial sarcoidosis cases. The recruitment methods of these studies have been previously described. The German study population used for validation of results consisted of 711 sarcoidosis cases and 1083 controls from a previously described sample.[6] All studies underwent human subjects' review, with the original informed consent documents specifying permission to use data and genetic material for future studies.

The characteristics of the study populations are summarized in Table 5. Briefly, the sample size was 2941—68% female and 73% African American. Sarcoidosis cases had their diagnoses confirmed by either: (1) histological confirmation with evidence of disease involvement in either the thorax or two or more other organ systems; or (2) characteristic chest radiographs (bilateral symmetrical hilar adenopathy) plus erythema nodosum or without diagnosis of another explanatory condition after a minimum of 2 years of follow-up.

A subset of African-American participants ($n = 954$) also had chest radiographic data categorized according to the four-stage Scadding scoring system. At a follow-up of 2 years or greater from diagnosis, radiographic stage was categorized as 'resolved' (normal or Scadding stage 0), 'persistent stages I–III' or 'persistent stage IV'.

Selection of tag SNPs and genotyping

Tagging SNPs were selected from the catalogs of the International HapMap Project and the 1000 Genomes Project. Using a multi-population tagging approach,[34] 291 SNPs were selected to identify variation present at ≥1% frequency in African Americans within the region containing *ANXA11* region on chromosome 10 (between base pairs 81 875 020–81 974 820) were selected and genotyped using an Illumina (San Diego, CA, USA) iSelect custom genotyping assay. Illumina quality scores (lower limit ≥0.4) were used to predict which SNPs were most likely to result in successful genotyping and to prioritize high-scoring SNPs as tags. Of the 291 SNPs assayed within the region, 209 produced analyzable genotypes. Individual SNPs were removed if > 10% of data was missing. Hardy–Weinberg equilibrium was tested by Fisher's exact test in a set of unrelated African American controls using PEDSTATS;[35] SNPs with Hardy–Weinberg equilibrium *P*-values < 0.001 were removed from further analyses. Genetic marker data on eight additional *ANXA11* SNPs and 13 737 SNPs spanning the HLA region on chromosome 6 (from base pair position 27 to 34 Mb) from a previous GWAS using the Illumina OmniQuad was also available for these samples.[9] These data were used to confirm the association between rs1049550 and sarcoidosis risk, and to explore interactions between rs1049550 and variation across the *HLA* region. Genotyping of rs61860052 in the German study population was conducted using Taqman technology (Applied Biosystems, Carlsbad, CA, USA).

Statistical analyses

To account for relatedness and potential population stratification between sarcoidosis cases and controls, our primary analysis was based on univariate SNP association tests conducted using the ROADTRIPS statistic of Thorton and McPeek,[36] which combines data from both unrelated and related individuals to test allelic association. The ethnicity-specific ROADTRIPS *P*-values were combined via inverse normal meta-analysis accounting for the direction of effect and weighted by the study sample size using the meta-analysis program METAL[37] to test for cross-ethnicity association. In addition, OR estimates and control for confounding (sex and individual percent African ancestry in African Americans; sex in European Americans) were performed using generalized estimating equations, fit to a logistic model treating each family as a cluster and utilizing an independent correlation structure.[38] The additive genetic model was used to estimate the OR associated with each SNP in the multivariate model. The independent effect of SNPs in the *ANXA11* region was assessed by forward-stepwise logistic regression, where *ANXA11* rs1049550 was fixed in the model.

In the subset of African Americans that had radiographic data and a minimum of 2 years of follow-up, single SNP models were stratified on Scadding stage (resolved, persistent stages I–IV, and persistent stage IV), comparing each Scadding stage strata with controls. These analyses were restricted to those SNPs found to be independently associated with sarcoidosis risk in African Americans using the forward-stepwise logistic modeling procedure. SNP–SNP interactions were modeled as a multiplicative interaction term in logistic regression models fit using generalized estimating equations, with single SNP main effects retained in the model. A Wald test was used to estimate the statistical significance of the interaction. Ethnicity-specific multiplicative interaction terms were combined via meta-analysis[37] accounting for the respective standard errors to determine the cross-ethnic effect of interaction between SNP rs1049550 in *ANXA11* and SNPs within the HLA region. Except where otherwise noted, all analyses were carried out using the R programming language (version 2.10.1, R Foundation for Statistical Computing, Vienna, Austria).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Primary support for this work was from research grants awarded to CGM (1RC2HL101499) and BAR (R56-AI072727 and R01-HL092576).

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Table 1

SNPs in the *ANXA11* region associated with risk of sarcoidosis

SNP[d]	Gene[d]	Alleles[a]	AA			EA			r^2 with rs1049550	
			Frequency[b] Control	Case	*P*-value[c]	Frequency[b] Control	Case	*P*-value[c]	AA	EA
rs4399277		A/G	0.09	0.11	0.018	0.07	0.06	0.499	0.03	<0.01
rs2789697	*PLAC9*	G/C	0.18	0.16	0.007	0.45	0.41	0.102	0.63	0.66
rs3762097	*PLAC9*	A/C	0.18	0.15	0.002	0.43	0.38	0.069	0.86	0.83
rs2789679		T/A	0.18	0.16	0.004	0.43	0.37	0.039	0.88	0.87
rs1953600		A/G	0.19	0.16	0.002	0.43	0.37	0.028	0.87	0.90
rs79622405		A/G	0.07	0.06	0.041	<0.01	<0.01	—	0.02	—
rs2789686	*ANXA11*	A/G	0.29	0.31	0.009	0.12	0.15	0.043	0.09	0.10
rs1802932	*ANXA11*	G/A	<0.01	<0.01	—	0.01	0.02	0.046	—	0.01
rs1079242	*ANXA11*	G/A	0.21	0.18	0.002	0.52	0.45	0.015	0.80	0.62
rs2573346	*ANXA11*	A/G	0.16	0.14	0.019	0.43	0.37	0.032	0.70	0.90
rs2784773	*ANXA11*	A/G	0.49	0.50	0.037	0.36	0.41	0.031	0.18	0.37
rs114637323	*ANXA11*	A/G	0.03	0.04	0.010	<0.01	<0.01	—	<0.01	—
rs7067644	*ANXA11*	G/A	0.43	0.42	0.460	0.61	0.56	0.032	0.33	0.46
rs1049550	*ANXA11*	C/T	0.17	0.15	0.001	0.42	0.36	0.012	1	1
rs3851054	*ANXA11*	A/G	0.25	0.23	0.003	0.48	0.43	0.039	0.67	0.80
rs116293236	*ANXA11*	G/A	0.03	0.04	0.010	<0.01	<0.01	—	<0.01	—
rs79199495	*ANXA11*	A/G	0.05	0.06	0.046	<0.01	<0.01	—	0.01	—
rs7901057	*ANXA11*	G/A	0.03	0.04	0.024	<0.01	<0.01	—	<0.01	—
rs7913325	*ANXA11*	G/A	0.12	0.13	0.037	0.05	0.07	0.191	0.03	0.04
rs2819947	*ANXA11*	A/G	0.26	0.24	0.035	0.42	0.36	0.007	0.66	1
rs2819945	*ANXA11*	G/A	0.35	0.33	0.350	0.56	0.49	0.006	0.45	0.58
rs2573375	*ANXA11*	C/G	0.18	0.16	0.001	0.42	0.36	0.006	1	1
rs61860052	*ANXA11*	A/C	0.02	0.01	0.035	0.11	0.09	0.240	<0.01	0.07
rs2253137		G/A	0.21	0.24	0.002	0.17	0.18	0.850	0.07	0.13
rs112535453		A/C	0.11	0.10	0.045	<0.01	<0.01	—	0.12	—

Abbreviations: AA, African American; ANXA11, annexin A11; EA, European American; SNP, single-nucleotide polymorphism.

[a] The alleles at each SNP correspond to the minor/major allele among African American sarcoidosis controls.

[b] Allele frequencies correspond to the minor allele among African American controls.

[c] SNPs with ROADTRIPS allelic association test *P*-values<0.05.

[d] SNPs are listed in ascending order with regard to base pair position in the hg19 build of the human genome. Gene designations were given if the SNP fell within the boundaries 5' and 3' boundaries of a gene, as defined by the largest transcript recorded for the gene within the NCBI's RefSeq database. SNPs without a gene designation are inter-genic.

Table 2

Multivariable ANXA11 SNP[a] model for sarcoidosis risk in African Americans

SNP	Alleles[b]	OR[c]	95% CI	*P*-value
rs1049550	C/T	0.84	0.72–0.98	0.032
rs61860052	A/C	0.62	0.40–0.97	0.038
rs4399277	A/G	1.31	1.06–1.63	0.014

Abbreviations: ANXA11, annexin A11; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a]The resulting multi-SNP model was determined by forward-stepwise selection, after fixing rs1049550 in the model. SNPs were included and retained in the model if the robust Wald statistic *P*-value was <0.05.

[b]The alleles at each SNP correspond to the minor/major allele among African American sarcoidosis controls.

[c]OR for each SNP with respect to the minor allele.

Table 3

OR estimates for ANXA11 SNPs associated[a] with sarcoidosis in African Americans, stratified by radiographic evidence of disease ≥ 2 years of follow-up

	rs1049550[b]			rs61860052[b]			rs4399277[b]		
Radiographic phenotype	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**	**OR**	**95% CI**	***P*-value**
Controls	1	Ref	Ref	1	Ref	Ref	1	Ref	Ref
Resolved	0.82	0.64–1.06	0.13	0.66	0.35–1.25	0.20	1.34	0.98–1.84	0.07
Persistent									
All	0.79	0.65–0.95	0.02	0.62	0.34–1.11	0.20	1.32	1.01–1.72	0.04
Stage IV	0.85	0.62–1.16	0.31	0.39	0.13–1.22	0.11	1.52	1.03–1.22	0.03

Abbreviations: *ANXA11*, annexin A11; CI, confidence interval; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] SNPs found to be conditionally independent and significantly associated with sarcoidosis (that is, SNPs from Table 2).

[b] The additive OR for each SNP correspond to increasing allele count for the C allele for rs1049550, the A allele for rs61860052, and the A allele for rs439927. All ORs were adjusted for sex and percent African ancestry.

Table 4

SNP–SNP interaction and sarcoidosis risk: ORs at ANXA11 rs1049550 stratified by HLA–DRA rs9268839[a] genotype and ethnicity

	HLA–DRA rs9268839 genotype						*P*-value for SNP–SNP[c]
	AA		AG		GG		
	N[b]	OR (95% CI)	N[b]	OR (95% CI)	N[b]	OR (95% CI)	
ANXA11 rs1049550 genotype							
African American							
TT	571/321	1 (ref)	294/235	1 (ref)	20/37	1 (ref)	0.004
TC	191/149	0.70 (0.54–0.90)	109/116	0.77 (0.56–1.05)	15/13	3.12 (1.07–9.04)	
CC	13/13	0.54 (0.25–1.15)	16/7	2.05 (0.79–5.31)	3/2	7.21 (0.51–103.08)	
European American							
TT	83/27	1 (ref)	91/68	1 (ref)	15/20	1 (ref)	0.001
TC	71/43	0.54 (0.30–0.97)	103/100	0.77 (0.50–1.17)	20/30	0.91 (0.37–2.23)	
CC	23/32	0.23 (0.12–0.47)	31/24	0.97 (0.52–1.81)	9/6	1.87 (0.53–6.65)	

Abbreviations: ANXA11, annexin A11; CI, confidence interval; *HLA*, human leukocyte antigen; OR, odds ratio; SNP, single-nucleotide polymorphism.

[a] SNP rs9268839, located ~16 kb downstream of *HLA–DRA*, is univariately associated with risk of sarcoidosis in both African Americans (OR = 0.71; 95% CI = 0.61–0.83; $P = 9.8*10^{-6}$) and European Americans (OR = 0.67; 95% CI = 0.54–0.84; $P = 3.7*10^{-4}$).

[b] *N* corresponds to the number of case/control individuals for each genotype by ethnicity.

[c] *P*-value for the interaction corresponds to the Wald test of the multiplicative interaction term (additive model) from the standard unconditional logistic regression model fit using generalized estimating equations.

Table 5

Demographic and clinical characteristics of the study sample

	African American (*n* = 2141)		European American (*n* = 800)	
	Cases (*n* = 1242)	**Controls (*n* = 899)**	**Cases (*n* = 447)**	**Controls (*n* = 353)**
Male, *n* (%)	317 (25.5)	281 (31.3)	192 (43.0)	148 (41.9)
Age[a]	36.6 (9.7)	50.4 (14.7)	42.6 (10.8)	43.7 (10.5)
Percent individual African ancestry[a]	82.5 (10.5)	81.4 (11.7)	—	—
Radiographic phenotype[b] (%)				
Resolved	304 (31.9)	—	40 (35.1)	—
Persistent				
All	650 (68.1)	—	74 (64.9)	—
Stages I–III	462 (80.3)	—	65 (87.8)	—
Stage IV	188 (19.7)	—	9 (12.2)	—

[a] Mean (s.d.).

[b] Total with 2-year follow-up chest X-ray and Scadding stage data: African American, *n* = 954; European American, *n* = 114.