**Genome-Wide Association Study of African and European Americans Implicates Multiple Shared and Ethnic Specific Loci in Sarcoidosis Susceptibility**

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**Abstract**

Sarcoidosis is a systemic inflammatory disease characterized by the formation of granulomas in affected organs. Genome-wide association studies (GWASs) of this disease have been conducted only in European population. We present the first sarcoidosis GWAS in African Americans (AAs, 818 cases and 1,088 related controls) followed by replication in independent sets of AAs (455 cases and 557 controls) and European Americans (EAs, 442 cases and 2,284 controls). We evaluated >6 million SNPs either genotyped using the Illumina Omni1-Quad array or imputed from the 1000 Genomes Project data. We identified a novel sarcoidosis-associated locus, NOTCH4, that reached genome-wide significance in the combined AA samples (rs715299, $P_{AA-meta} = 6.51 \times 10^{-10}$) and demonstrated the independence of this locus from others in the MHC region in the same sample. We replicated previous European GWAS associations within HLA-DRA, HLA-DRB5, HLA-DRB1, BTNL2, and ANXA11 in both our AA and EA datasets. We also confirmed significant associations to the previously reported HLA-C and HLA-B regions in the EA but not AA samples. We further identified suggestive associations with several other genes previously reported in lung or inflammatory diseases.

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**Introduction**

Sarcoidosis is a systemic disease characterized by granulomatous inflammation that primarily affects the lungs, but can affect any organ [1,2,3]. While the etiology of this disease remains elusive, the pathophysiology likely involves a dysregulated immune response to environmental agents in a genetically susceptible host. Several environmental exposures have been associated with sarcoidosis including mold, inorganic particles, and insecticides [4,5,6]. A significant genetic component to sarcoidosis is supported by a 2.5 fold elevated disease risk in siblings and parents of cases [7] as well as several environmental exposures [8,9,10,11,12].

Sarcoidosis impacts individuals of all races, ages and genders [13], but in the U.S. is most frequent in AAs [14,15], with disease onset peaking between the ages of 20 and 39 years [16]. The AA population is more commonly affected than EAs [16,17,18,19], with a three-fold higher lifetime risk (2.4%) and age-adjusted annual incidence (35.5 per 100,000) compared to EAs (0.85% and 10.9 per 100,000, respectively). AA patients have higher disease severity and more extra-thoracic involvement than EA patients and are less likely to have disease that resolves [20]. Ethnicity specific prevalence and severity support the involvement of genes and further suggest ethnicity-specific genetic risk profiles.

Genetic associations with specific HLA alleles and sarcoidosis have repeatedly been reported [21,22,23,24]. Heterogeneity of these HLA effects in sarcoidosis across ancestries was observed in the ACCESS study [23] suggesting that while the *HLA-DRB1*1101 allele was associated with sarcoidosis in AAs and EAs, the *HLA-DRB1*1501 allele was associated with sarcoidosis only in EAs [23]. Recent studies have reported additional susceptibility loci including *BTNL2* [9,25,26] in both EAs and AAs.
AAs, and ANXI11 [11] and RAB23 [27] in Germans. The first genome-wide linkage study of AA sarcoidosis families performed by our group found prominent linkage signals on chromosome 5, at 5q11.2, 3p13, and 5q31 [10]. Our admixture study confirmed the latter two of these effects and found regions on chromosomes 6p22.3 and 17p13.3-17p13.1 associated with increased African ancestry [28]. Based on clear evidence of the involvement of genes in the onset and manifestation of sarcoidosis, we sought to confirm sarcoidosis genetic risk loci reported in association scans of European populations and to identify novel risk loci by conducting the first genome-wide association study (GWAS) of sarcoidosis in an American population. We present results from a family-based discovery cohort of AAs as well as two independent replication sets of AA cases and controls and EA cases and controls.

Results

Genome-wide Association Scan of AA Discovery Set

A total of 864,829 single-nucleotide polymorphisms (SNPs) in our AA discovery set passed quality control assessment (Materials and Methods, Figure 1, Table 1). To increase the density of SNPs to be tested for association, we performed genotype imputation across the genome with the 1000 Genomes Project Phase I haplotypes as reference (Materials and Methods). The GWAS of the AA discovery set demonstrated no evidence for inflation of the test statistics (genomic control inflation factor $k_{GC} = 1.030$, Figure S1). A haplotype-based association analysis of the test statistics in this dataset ($l^2 = 0.003$) also failed to detect associations from common alleles with odds ratios $\geq 1.5$ (Figure S2). We only found variants within previously reported MHC Class II genes [11,22] exceeding genome-wide significance in this dataset (Figure 2A, Figure 3A, Table S2); HLA-DR4, $P_{AA-disco} = 1.40 \times 10^{-6}$, was the most significant signal (Table S3). Additional MHC regions were also identified such as $P_{AA-disco} = 1.74 \times 10^{-6}$ and $P_{AA-disko} = 1.56 \times 10^{-6}$ (Table S3). The subsequent (and final) step adding this HLA-DR4 SNP (rs9273230) as the covariate resulted in diminished association signals for the remaining significant SNPs within the MHC class II genes ($P_{conditional} = 0.014$), whereas NOTCH4 remained significant ($P_{conditional} = 8.03 \times 10^{-6}$) (Table S3). While the $P$-value for NOTCH4 did not retain the GWAS threshold of $5 \times 10^{-8}$ after rigorous conditioning, it remains the only significant effect well exceeding the suggestive level of association. It suggests that the observed signal within NOTCH4 is independent of the evaluated SNPs within the MHC Class II genes. These analyses also showed the existence of multiple independent signals within this MHC region (Table 2).

Confirmation of Previously Reported SNPs Associated with Sarcoidosis in the Combined AA Datasets

Three significant SNPs reported in the previous German GWAS in the MHC region ($P < 5 \times 10^{-8}$) [11] were also replicated in our combined AA datasets ($P_{AA-met} = 1.40 \times 10^{-6}$; rs9268553, $P_{AA-disco} = 7.40 \times 10^{-4}$; and rs615672, $P_{AA-disko} = 2.60 \times 10^{-8}$, Table 3). The previously reported peak SNP within BTN2L2 ($P_{AA-met} = 0.024$, Table 3). However, a SNP with 4 kb upstream of rs2076530, rs9268482, was suggestive of association ($P_{AA-met} = 6.32 \times 10^{-6}$, Table 3). Interestingly, we also identified a suggestive association at a BTN2L2 coding-synonymous SNP, rs9268480 ($P_{AA-met} = 1.03 \times 10^{-5}$), only 28 bp upstream of rs2076530 and in high LD with rs9268482 ($r^2 = 0.96$). Since BTN2L2 is only 170 kb apart from NOTCH4, we sought to assess whether the signal within NOTCH4 is independent of the signal within BTN2L2 using conditional association analyses. When adjusting for one of those associated BTN2L2 SNPs (rs9268482), we found NOTCH4 remained significant ($P_{conditional} = 2.86 \times 10^{-6}$). On the other hand, after adjusting for the NOTCH4 SNP, we still observed a significant residual signal at the BTN2L2 SNP (rs9268482, $P_{conditional} = 1.26 \times 10^{-5}$). These indicated the signal within NOTCH4 is also independent of the BTN2L2 signal.

We saw modest association with two other previously reported susceptibility genes: ANXI11 [11] and RAB23 [27]. A non-synonymous SNP within ANXI11, rs1049550, was associated with sarcoidosis in our combined AA datasets at $P_{AA-met} = 8.46 \times 10^{-4}$ (Table 3). A similar modest association was seen with a non-synonymous SNP within RAB23 (rs1040461, $P_{AA-met} = 8.04 \times 10^{-3}$, Table 3). We did find suggestive evidence of association on 5q11.2 (peak signal at rs16137605 within a region between $SNX18$ and $ESM1$, $P_{AA-met} = 3.09 \times 10^{-5}$) a region identified in our previous linkage and fine-mapping studies [10,28,30].
Genome-wide Association Scan of EA Dataset

We found 682,921 genotyped SNPs passed quality control measures in our EA dataset (Materials and Methods, Figure 1, Table 1). After performing imputation with the 1000 Genomes Project haplotypes, the SNP-sarcoidosis association calculated using logistic regression of the EA dataset showed little evidence for inflation of the test statistics ($\lambda_{GC} = 1.027$, Figure S1). This dataset also had good statistical power ($\alpha = 5 \times 10^{-8}$) to detect associations from common alleles with odds ratios $1.5$ (Figure S2). We observed genome-wide significance SNPs within previously reported MHC genes [9,11,24] including $\text{HLA-C}$ (peak signal at rs6457375, $P_{EA} = 1.98 \times 10^{-19}$), $\text{HLA-B}$ (peak signal at rs2596475, $P_{EA} = 3.82 \times 10^{-19}$), and $\text{HLA-DRB5}$ (peak signal at rs17203612, $P_{EA} = 1.82 \times 10^{-19}$) (Figure 2C, Figure 3D, Table 2, Table S2). However, we did not find any variant within $\text{NOTCH4}$ passed genome-wide significance in this dataset (Figure S3).

Stepwise conditional association analyses further demonstrated two independent signals exist within this region tagged by rs6457375 ($\text{HLA-C}$) and rs17203612 ($\text{HLA-DRB5}$) (Table S4).

Confirmation of Previously Identified Loci in EA Dataset

We replicated significant SNPs from the German GWAS [11] in the EA dataset including rs7194 ($\text{HLA-DRA}$, $P_{EA} = 1.26 \times 10^{-4}$), rs9268853 ($\text{HLA-DRB5}$, $P_{EA} = 9.79 \times 10^{-4}$), rs615672 ($\text{HLA-DRB1}$, $P_{EA} = 8.00 \times 10^{-4}$), and rs1049550 ($\text{ANXA11}$, $P_{EA} = 8.33 \times 10^{-3}$) (Table 3). We also replicated the $\text{BTNL2}$ SNP, rs2076530 [9,11,25], in our EA dataset ($P_{EA} = 4.19 \times 10^{-6}$, Table 3). We did not, however, confirm the $\text{RAB23}$ association [27] in this dataset ($P_{EA} = 0.418$, Table 3).

**Table 1. Sample summary before and after quality control (QC).**

| Characteristic                  | African American before QC | African American after QC | European American before QC | European American after QC |
|--------------------------------|-----------------------------|---------------------------|----------------------------|----------------------------|
| Cases                          | 1487                        | 818                       | 455                        | 1273                       |
| Controls                       | 1504                        | 908                       | 577                        | 1465                       |
| External Controls              | 180*                        | 180                       | 0                          | 180                        |
| Unknown Affection Status       | 2                           | 0                         | 0                          | 0                          |
| Male                           | 889                         | 575                       | 244                        | 819                        |
| Female                         | 2264                        | 1331                      | 768                        | 2099                       |
| Unknown Gender                 | 20                          | 0                         | 0                          | 0                          |
| Total                          | 3173                        | 1906                      | 1012                       | 2918                       |

*Taken from the Illumina YRI-ASW iControlDB; 
175 Caucasian healthy controls from the Illumina iControlDB, 1047 controls from the dbGaP GENEVA Melanoma study, and 1886 controls from the dbGAP CIDR: NGRC Parkinson’s Disease Study.

Figure 1. A graphical overview of the GWAS datasets. (A–B) Summary of the AA (A) and EA (B) datasets. doi:10.1371/journal.pone.0043907.g001
Meta-analysis Results of All Datasets

Among regions that met genome-wide significance in the AA meta-analysis, we also found significant associations within HLA-DRA, HLA-DRB1, and HLA-DQA1 in the EA dataset (8.25 \times 10^{-10} \leq P_{EA} \leq 3.97 \times 10^{-8}, 3.77 \times 10^{-14} \leq P_{All-meta} \leq 7.23 \times 10^{-8}) (Figure 3E, Table S2). We found a weak association to the NOTCH4 SNP (rs715299) in the EA dataset (P_{EA} = 0.096), perhaps suggesting its ethnicity specific effect (the Cochran’s Q test of heterogeneity P = 0.064 and the inconsistency index I^2 = 63.60%, see Materials and Methods). Conversely, when evaluating regions reaching genome-wide significant in the EA dataset, variants within HLA-DRB5, HLA-DRB1, and HLA-DQA1 were also significant in the AA datasets (1.81 \times 10^{-7} \leq P_{AA-meta} \leq 1.28 \times 10^{-3}, 1.16 \times 10^{-11} \leq P_{All-meta} \leq 2.65 \times 10^{-12}, Table S2), whereas HLA-C and HLA-B were not (P_{AA-meta} \geq 0.575, Table S2).

Suggestive Association Regions

We observed multiple regions reached suggestive association (P_{All-meta} < 1 \times 10^{-8}) in the meta-analysis of all AA and EA datasets. These included variants within TRAK1, SLC44A4, GLI3-C7orf25, ATPA2, and TGM3 (Tables S5). We observed additional suggestive association regions (P < 1 \times 10^{-8}) that were unique to one ethnic group. For example, we identified variants with suggestive association within FHIT, PRDM1, FRMD3, DMBT1 and a region between ZSCAN2 and ALPK3 in the combined AA datasets only (Tables S5). We also observed suggestive association only in the EA dataset within CASP10, RARB, and NCR3 among others (Tables S5). Several of these suggestive effects fall within genes implicated in other lung or inflammatory diseases (Table S6).

Discussion

Previously reported GWASs of sarcoidosis have been limited to European (specifically German) samples. Ours is the first GWAS of sarcoidosis in Americans and, even more importantly, of AAs, the population most commonly and severely affected. Our results, while demonstrating some shared effects across ethnicities, strongly support the presence of ethnic specific genetic effects. We identified significant association between sarcoidosis and a previously unreported locus (NOTCH4) in our AA datasets. This association was determined to be independent of other neighboring MHC genes and is an attractive biological candidate. NOTCH4 encodes a member of the Notch family that is involved in...
in controlling cell fate decisions during developmental processes and regulating the activity of T cell immune responses [31,32]. The Notch signaling pathway also plays a role in endothelial cell differentiation, apoptosis and proliferation [33,34,35,36]. Further, NOTCH4 is highly expressed in the lung and may play a key role in the lung development and diseases such as asthma and lung arteriovenous shunts [37,38,39,40,41]. NOTCH4 has also been associated with neonatal lupus [42], multiple sclerosis [43], systemic sclerosis [44], and other immune-related disorders [45,46,47,48]. We also saw evidence of suggestive association of NOTCH4 in our EA dataset. While further studies are needed to define the role of NOTCH4 in the specific pathogenesis of sarcoidosis, a novel association to this gene is supported by previous expression and disease studies.

We replicated associations for several previously reported sarcoidosis susceptibility risk loci in our AA collection including MHC Class II region genes (HLA-DRA, HLA-DRB5, HLA-DRB1, and HLA-DQA1), BTNL2, RAB23, and ANXA11 [9,11,25,27,49]. These regions were also replicated in our EA dataset except for RAB23. It is known that the MHC Class II region plays a major role in immune-mediated disorders, including associations to celiac disease, insulin-dependent diabetes mellitus, rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus (SLE) [50,51]. Similarly, BTNL2, RAB23, and ANXA11 have been
Table 2: Regions of association meeting genome-wide significance and their most significant SNPs, grouped by sample.

| Gene/Allele | CHR/Bp (19) | Haplotype | African Americans | European Americans |
|-------------|-------------|-----------|-------------------|---------------------|
| HLA-DRA | 6 32,446,853 | rs17203612 | 0.270 0.64 2.66E-05 | 0.243 0.79 2.42E-02 |
| HLA-DQA1 | 6 32,642,794 | rs149288329 | 0.454 1.30 1.12E-05 | 0.480 1.52 8.14E-06 |
| NOTCH4 | 6 32,189,841 | rs715299 | 0.423 0.88 4.24E-01 | 0.403 1.17 9.06E-02 |
| HLA-C | 6 31,272,612 | rs6457375 | 0.50 1.58 1.98E-09 | 0.324 1.14 9.58E-02 |

Note: Stepwise conditional analysis results to identify independent signals within the MHC region can be found in Tables S3 and S4.

Suggested to play a role in T-cell activation [9], antibacterial defense processes [27], and apoptosis [11]. It is worth noting that we did not replicate the association with C10orf67 [12] as identified in a joint GWAS of German patients with either sarcoidosis or Crohn’s disease.

Additional regions with suggestive evidence of association in both AAs and EAs include TRAK1, SLCA4A4, GLI3-C7orf25, ATP8A2, and TGM3. While the biological relevance of most of these genes to sarcoidosis is still unknown, GLI3-C7orf25 and TGM3 may warrant further investigation. Although C7orf25 is a hypothetical gene with unknown function, GLI3 encodes zinc finger protein GlI3 that has a bipotential function as a transcriptional activator or repressor of the sonic hedgehog pathway [52,53]. This pathway contains RAB23 (discussed above) and has been suggested to play a role in the sarcoidosis pathophysiology [27]. TGM3 (Transglutaminase 3) encodes protein involved in the later stages of cell envelope formation in the epidermis and hair follicle [54] and has been associated with celiac disease [55,56] and psoriasis [57,58].

Despite the overlap of compelling signals across populations, we did find evidence of genetic heterogeneity between ethnic groups in this disease (see Tables 2 and 3). The previously reported MHC Class I region [24] including HLA-C and HLA-B (associated with psoriasis [59] and ankylosing spondylitis [60], respectively) was associated only in the EA dataset. Other noteworthy genes with suggestive association specific to EAs included CASP10, RARB, and NCR3. CASP10 (caspase 10) plays a role in apoptosis and has been associated with autoimmune lymphoproliferative syndrome [61] and non-Hodgkin lymphoma [62]. In addition, RARB (retinoic acid receptor beta) and NCR3 (natural cytotoxicity triggering receptor 3) have been associated with pulmonary function based on a recent GWAS of European Caucasians [63]. Suggestive associations specific to AAs include FHTT, FRMD3, DMBT1, and PRDM1. FHTT (fragile histidine triad) is involved in various intracellular functions and a putative tumor suppressor for various cancers including lung cancer [64,65]. FRMD3 (FERM domain containing 3) is over-expressed in normal human lung tissue compared with tissue from lung tumors of lung carcinoma patients suggesting its important role in the origin and progression of lung cancer [66]. DMBT1 (deleted in malignant brain tumors 1) is overexpressed in epithelial cells [67] and has been found associated with ulcerative colitis [68] and Crohn’s disease [67,69]. PRDM1 (PR domain containing protein 1) plays a role as a repressor of beta-interferon gene expression [70] and had been associated with rheumatoid arthritis [71], inflammatory bowel disease (IBD) [72,73], and SLE [74,75]. We also observed variants with suggestive associations specific to AAs in a region containing ZSCAN2, SCLN2, WDR73, NMB, SECL1A, C7orf25, and ALPK3 as well as a region identified in our linkage studies [10,28,30] on 5q11.2 (a region between NXK18 and ESM1). However, the actual biological functions of these genes are largely unknown.

In summary, this is the first report of GWAS in an American sample and the first report of a significant association between sarcoidosis and NOTCH4. We have replicated several previously reported sarcoidosis susceptibility loci in both our EA and AA samples as well as report several biologically plausible effects at loci with suggestive statistical evidence. We report sarcoidosis associations both shared between ethnicities as well as those unique to either our AA or EA dataset, supporting genetic heterogeneity of this disease. The presence of genetic heterogeneity may well serve as a useful tool in the isolation of the causal variants associated with this disease as it has in other complex disorders [76,77]. Finally, this study demonstrates both the usefulness of and need for
genetic studies of sarcoidosis in diverse populations and further elucidates potential pathogenic mechanisms of this disease. Future replication, sequencing and functional studies are required to further elucidate the causal variants that may underlie these associations as well as to discover rare variants that may have yet to be identified.

Materials and Methods

Ethics Statement

The study and sample collection were approved by the Institutional Review Board (IRB) at all participating institutions including A Case Control Etiologic Study of Sarcoidosis (ACCESS) Group, Sarcoidosis Genetic Analysis (SAGA) study, Henry Ford Health System in Detroit, Michigan, and Oklahoma Medical Research Foundation (OMRF), Oklahoma City, Oklahoma, Institutional Review Boards (IRBs). Only individuals who signed informed consent forms were included in this study. No minors or children were involved in our study.

Subjects

Our AA sample collection, which comprises 1487 cases and 1504 controls (Figure 1, Table 1), was taken from an extensive cohort of AA sarcoidosis patients, family members and controls assembled from 1) case-control pairs collected as a part of a 10 center collaborative study (ACCESS Group) [78], 2) the SAGA sample ascertained through affected sib pairs [79], 3) a nuclear family-based sample ascertained through single sarcoidosis-affected offspring from the Henry Ford Health System in Detroit, Michigan [80], and 4) healthy controls from the OMRF Lupus Family Registry and Repository (LFRR) [81]. The AA cases and their family members were grouped into a discovery set of 818 cases and 908 related and unrelated controls and the other 455 independent cases and 557 independent controls were selected for a replication set after applying quality control measures as described below (Figure 1, Table 1). In addition, genotype data from 180 HapMap controls from Yoruba in Ibadan, Nigeria (YRI) and of African ancestry in Southwest USA (ASW) were obtained from the Illumina HumanOmn1-Quad iControlDB (http://www.illumina.com/science/icontroldb.ilmn) and included into the control group of the AA discovery set, as is common practice in order to increase statistical power [82,83,84].

Genotyping and Quality Control

Genotyping was performed at the OMRF using the Illumina HumanOmn1-Quad array for ~1.1M variants across the genome. SNPs had to meet the following quality control criteria for inclusion for each population: well-defined cluster plots by visual inspections, call rate >95%, minor allele frequency >0.01, Hardy-Weinberg proportion tests \( P > 0.0001 \) in cases and \( P > 0.001 \) in controls, and case-control differences in missingness \( P > 0.001 \). Copy number variations, X, Y, XY, and mitochondrial chromosomes were not included in the analysis. A total of 864,829 and 682,921 SNPs passed our quality controls in the AA discovery and
replication sets and the EA dataset, respectively. We found 657,350 successfully genotyped SNPs that overlap between the panels. Samples were removed from analysis if they were determined to be a duplicate of another sample, cryptic relatedness in the independent datasets (the proportion of alleles shared identical by descent >0.25), exhibited extreme heterozygosity (>5 standard deviations from the mean), demonstrated either outlying principal component values of population membership calculated by EIGENSOFT 3.0 [85] or global ancestry estimates calculated by ADMIXMAP [86,87], or revealed discrepancies between reported gender and genetic data (Table S1). For the EA dataset, we assigned to each sarcoidosis case the five best-matched controls as determined by identity-by-state (IBS) allele sharing using PLINK v1.07 [88] resulting in a large drop-out of external controls in the EA dataset.

Imputation Method

Imputation was performed in each population at 5 Mb bins across the genome using the IMPUTE2 program [89,90]. The 1000 Genomes Project Phase I data release [June 2011], which contains haplotypes derived from 1,094 individuals from Africa, Asia, Europe, and the Americas, was used as the reference [89,90]. IMPUTE2 estimated the posterior probabilities for the three possible genotypes (i.e. AA, AB, and BB). The posterior probabilities were then converted to the most likely genotypes with a threshold of 0.9. Imputed SNPs with either low imputation accuracy (information measure <0.5 and the average maximum posterior genotype call probability <0.9) and that failed the SNP quality control standards described above were removed in order to minimize false positives. After imputation, 10,948,298 SNPs in the AA discovery set, 11,160,451 SNPs in the AA replication set, and 6,620,482 SNPs in the EA replication set passed quality control measures for analysis.

Association Analyses

Because our discovery set contained related individuals, association analysis to any single marker in this set was performed using the Efficient Mixed-Model Association eXpedited (EMMAX) software [91,92]. EMMAX was chosen because it implements a variance component approach in the linear mixed-model that simultaneously adjusts for both pairwise genetic relatedness between individuals and corrects for population stratification using an empirical kinship matrix based on the proportion of alleles at all genome-wide SNPs shared identical-by-state between all pairs of individuals in the study [91]. We assumed an additive model [91,92] and adjusted the statistics for gender. Since EMMAX does not calculate odds ratios (ORs), we estimated these using logistic regression as implemented in PLINK using independent samples (480 cases and 367 controls) ascertained from the AA discovery set. The association analyses of the independent sets of AAs and EAs were calculated using logistic regression in PLINK. We assumed the additive genetic model and adjusted the statistics for gender and the first five principal components of each population (calculated using EIGENSOFT 3.0). Meta-analyses were performed using the weighted \( \frac{Q}{F} \) score method that accounts for the direction of effects and sample-size as implemented in METAL [93]. Both the Cochran’s Q test statistic and \( \frac{F}{Q} \) index were used to test for heterogeneity in the meta-analysis of all samples. The Cochran’s Q test calculates the weighted sum of the squared deviations between each study effects and the overall effect across studies [94], whereas the \( \frac{F}{Q} \) index quantifies the percentage of inconsistency across studies due to heterogeneity rather than by chance [95]. The Q test with \( P<0.05 \) or \( \frac{F}{Q} >50\% \) indicates the presence of heterogeneity. Stepwise conditional association analysis in AAs was conducted for SNPs with \( P<5 \times 10^{-8} \) using EMMAX adjusting for gender and SNPs of interest, a SNP added at a time. We required a SNP threshold of \( P<5 \times 10^{-8} \) to be considered significantly associated and \( P<1 \times 10^{-4} \) to be considered suggestively associated with sarcoidosis [96,97,98].

The power calculations for different minor allele frequencies and odds ratios for each dataset were performed using the Genetic Power Calculator program [99] and have been summarized in Figure S2. The assumptions are a disease prevalence of 0.05%, complete linkage disequilibrium between SNP and predisposing loci, an additive genetic model and a type I error rate \( \alpha = 5 \times 10^{-5} \). To present power curves that are comparable across sets, we used a power calculator that assumes independence, but adjusted the analysis of the AA discovery set (family-based set) assuming a familial correlation of 0.25 since most pairs are siblings (and thus smaller equivalent count or 75% of the total cases and controls in this set).

Supporting Information

Figure S1 The quantile-quantile (Q-Q) plots of the observed and expected distributions of \( P \)-values. (A–C) The Q-Q plots for (A) the AA discovery set (genomic control inflation factor \([\lambda_{GC} = 0.980\]), (B) the AA replication set \([\lambda_{GC} = 1.030\]), and (C) the EA dataset \([\lambda_{GC} = 1.027\]).

Figure S2 Power calculation plots of the GWAS datasets. (A–C) Power calculation plots for the AA discovery set (A), the AA replication set (B), and the EA dataset (C).

Figure S3 Regional association plots of SNP-sarcoidosis association test results within NOTCH4. (A–D) Association results in the AA discovery set (A), AA replication set (B), a meta-analysis between the AA discovery and AA replication sets including the LD \( (D') \) plot (C), and the EA dataset including the LD \( (D') \) plot (D). Each SNP is colored according to its LD \( (D') \) with the top SNP. The blue solid line denotes the recombination rate.

Table S1 Summary of dropped samples after QC.

Table S2 Association results with \( P<5 \times 10^{-8} \) in either dataset.

Table S3 Stepwise conditional analysis in AA samples for SNPs in the MHC region with \( P<5 \times 10^{-8} \).

Table S4 Stepwise conditional analysis in EA samples for SNPs in the MHC region with \( P<5 \times 10^{-8} \).

Table S5 Association results with \( P<1 \times 10^{-4} \) in either dataset.

Table S6 Shared or Ethnic Specific Suggestive Association Regions supported by the heterogeneity test results and list of inflammatory or lung diseases associated with these regions.
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Author Contributions
Conceived and designed the experiments: IA MCI BAR KLM RPK JBH CGM. Performed the experiments: IA CPL AA KMK. Analyzed the data: IA CPL JJH AML. Wrote the paper: IA

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