Association of genetic variants in RAB23 and ANXA11 with uveitis in sarcoidosis

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Purpose: Uveitis occurs in a subset of patients with sarcoidosis. The purpose of this study was to determine whether genetic variants that have been associated previously with overall sarcoidosis are associated with increased risk of developing uveitis.

Methods: Seventy-seven subjects were enrolled, including 45 patients diagnosed with sarcoidosis-related uveitis as cases and 32 patients with systemic sarcoidosis without ocular involvement as controls. Thirty-eight single nucleotide polymorphisms (SNPs) previously associated with sarcoidosis, sarcoidosis severity, or other organ-specific sarcoidosis involvement were identified. Allele frequencies in ocular sarcoidosis cases versus controls were compared using the chi-square test, and p values were corrected for multiple hypotheses testing using permutation. All analyses were conducted with PLINK.

Results: SNPs rs1040461 and rs61860052, in ras-related protein RAS23 (RAB23) and annexin A11 (ANXA11) genes, respectively, were associated with sarcoidosis-associated uveitis. The T allele of rs1040461 and the A allele of rs61860052 were found to be more prevalent in ocular sarcoidosis cases. These associations remained after correction for the multiple hypotheses tested (p=0.01 and p=0.02). In a subanalysis of Caucasian Americans only, two additional variants within the major histocompatibility complex (MHC) genes on chromosome 6, in HLA-DRB5 and HLA-DRB1, were associated with uveitis as well (p=0.009 and p=0.04).

Conclusions: Genetic variants in RAB23 and ANXA11 genes were associated with an increased risk of sarcoidosis-associated uveitis. These loci have previously been associated with overall sarcoidosis risk.

Sarcoidosis is a multisystem disease that is characterized histologically by the presence of non-caseating epithelioid granulomas [1]. Uveitis is the most common ocular manifestation, seen in 25–50% of patients [2]. About one quarter of patients with ocular involvement will develop severe and sight-threatening complications.

The etiology of sarcoidosis is poorly understood. There is substantial evidence for a genetic predisposition to sarcoidosis overall. Monozygotic twins are more often concordant for the disease than dizygotic twins [3,4]. Familial clustering occurs in approximately 5–16% of patients [5]. The risk of sarcoidosis is increased 2.5-fold for siblings and parents of a patient with sarcoidosis [6]. Differences also exist in the prevalence and clinical manifestations of the disease in different geographic areas and racial groups which could be due to genetic differences [7].

Sarcoidosis genetic studies have yielded several polymorphisms that have now been consistently associated with increased risk of sarcoidosis. The first reported possible association between sarcoidosis and a specific gene involved class I human leukocyte antigen (HLA-B; Gene ID 3106; OMIM 142830) and was published more than 30 years ago [8]. Since then, HLA class II antigens (HLA-DRB1 [Gene ID 3123; OMIM 142857] and -DQB1 [Gene ID 3119; OMIM 604305]) have been consistently linked to the susceptibility and prognosis of the disease [9,10]. To date, eight genome-wide association studies (GWASs) have correlated single nucleotide polymorphisms (SNPs) with sarcoidosis; these studies were performed in European and African American populations [6,11-15]. Non-HLA genes that have been associated in GWASs with sarcoidosis include butyrophilin-like 2 (BTLN2; Gene ID 56244; OMIM 606000), nucleotide-binding oligomerization domain 2 gene (NOD2; Gene ID 64127; OMIM 605956), notch homolog protein 4 (NOTCH 4; Gene ID 4855; OMIM 164951), ras-related protein RAS23 (RAB23; Gene ID 51715; OMIM 606144), and annexin A11 (ANXA11; Gene ID 311; OMIM 602572), among others. Polymorphisms in genes encoding cytokines (transforming growth factor-beta, interleukin-8, and tumor necrosis factor-alpha) and Toll-like receptor-4 have also been reported to be associated.
with more severe sarcoidosis disease course or particular extrapulmonary manifestations [16,17].

In contrast with genetic studies for overall sarcoidosis, few genetic studies specifically examined the commonly encountered phenotypic variant of ocular involvement in sarcoidosis. Thompson et al. found that a complement factor H (CFH; Gene ID 3075; OMIM 134370) variant, which has been shown to be strongly associated with age-related macular degeneration, was also associated with ocular sarcoidosis when subjects with ocular sarcoidosis were compared to sarcoidosis-free controls [18]. Spagnolo et al. reported that a polymorphism in heat shock protein-7 (HSP-70/HOM; Gene ID 3305; OMIM 140559) was associated with sarcoidosis-related uveitis when patients with sarcoidosis-related uveitis were compared to patients with sarcoidosis without uveitis and sarcoidosis-free controls [19]. Kim et al. investigated the association between 11 IL-23R (Gene ID 149233; OMIM 607562) SNPs and susceptibility to sarcoidosis in patients with sarcoidosis (58 with and 33 without uveitis) versus healthy controls [20]. Two of these SNPs (rs11465804 and rs11209026) were associated with the uveitis subgroup compared to healthy controls. None of these findings have been replicated in independent samples.

Sarcoidosis is a phenotypically heterogeneous disease, and genetic variation could explain the variability in organ-specific involvement [21]. We hypothesized that genetic variation may predispose certain patients with sarcoidosis to uveitis. The purpose of this study was to determine whether SNPs that have been associated previously with overall sarcoidosis, sarcoidosis severity, or non-pulmonary sarcoidosis also increase the risk of developing uveitis in patients with sarcoidosis.

METHODS

Participants: This genetic case–control study was approved by the Institutional Review Board at the Massachusetts Eye and Ear Infirmary (MEEI) and conformed to the tenets of the Declaration of Helsinki. This study adhered to the ARVO statement on human subjects. Written informed consent was obtained from all participants. Participants were enrolled from two sources: (1) the MEEI Uveitis Clinics and (2) the Partners HealthCare Biobank, a biorepository of consented patient DNA samples from patients seen at our affiliated general hospitals, the Massachusetts General Hospital and Brigham and Women’s Hospital. All patients had documentation of a complete ophthalmic examination and a thorough systemic evaluation before inclusion into the study.

Sarcoidosis definition: All participants included in the primary analysis had a diagnosis of sarcoidosis established by 1) histological confirmation with evidence of disease involvement in either the thorax or two more other organ systems or 2) characteristic chest radiographs (bilateral hilar lymphadenopathy) without diagnosis of another explanatory condition after a minimum of 2 years follow-up. Large GWASs and candidate gene studies for sarcoidosis have used these definitions [6,14,22,23]. As in these previous studies, we included patients who did not have tissue confirmation of sarcoidosis because an invasive diagnostic procedure is frequently deferred in patients with symmetric hilar lymphadenopathy [23].

Control definition: Sarcoidosis without uveitis: For the primary analysis, controls were defined as patients with sarcoidosis but without uveitis. Absence of uveitis was excluded by documentation of normal yearly eye examinations.

Case definition: Sarcoidosis with uveitis: For the primary analysis, cases were defined as patients with sarcoidosis-related uveitis. The definition of sarcoidosis-related uveitis was based on the criteria set forth by the International Workshop on Ocular Sarcoidosis (IWOS). The IWOS defined a uveitis that is compatible with sarcoidosis-related uveitis based on seven signs: 1) mutton-fat keratic precipitates (KPs)/small granulomatous KPs and/or iris nodules, 2) trabecular meshwork nodules and/or tent-shaped peripheral anterior synechiae, 3) vitreous opacities displaying snowballs/strings of pearls, 4) multiple chorioretinal peripheral lesions (active and/or atrophic), 5) nodular and/or segmental peri- phlebitis (± candlewax drippings) and/or retinal macroaneurysm in an inflamed eye, 6) optic disc nodule(s)/granuloma(s) and/or solitary choroidal nodule, and 7) bilaterality [24]. We included patients who met the criteria for either definitive or presumed sarcoidosis-related uveitis. A diagnosis of definitive sarcoidosis-related uveitis was made in the presence of a compatible uveitis with a confirmatory biopsy of any organ. Presumed sarcoidosis-related uveitis was defined by a compatible uveitis supported by bilateral hilar lymphadenopathy seen on chest imaging without a biopsy. The anatomic subtype of uveitis was determined according to the Standardization of Uveitis Nomenclature (SUN) criteria [25]. Presence versus absence of retinal vasculitis was also noted for each patient.

We chose the case and control definitions as detailed above because we felt these were the optimal definitions for achieving our main purpose which was to identify genes that convey risk specifically to uveitis in the setting of systemic sarcoidosis. Because some of the previous studies that have tried to identify genes for sarcoidosis-associated uveitis compared sarcoidosis-associated uveitis patients to
sarcoidosis-free controls [18-20], we also recruited sarcoidosis-free controls from the Partners HealthCare Biobank only for the purpose of trying to replicate this subset of original findings with optimal precision. These participants were identified as having no history of sarcoidosis and a documented normal eye examination on detailed electronic medical record review.

**Literature review:** To identify variants that have been previously associated with sarcoidosis, sarcoidosis severity, and/or other organ-specific sarcoidosis, we performed PubMed searches with the following criteria without a publication date or language limit. One PubMed search was performed using the following keywords to identify genome-wide association studies for sarcoidosis: (sarcoidosis OR sarcoid) AND [genome-wide association OR GWAS]. Previous candidate gene association studies were identified by the following PubMed search: [((genetics OR genetic OR gene OR polymorphism OR SNP OR allele OR genotype OR variant OR variation OR mutation)) AND (sarcoidosis OR sarcoid)]. SNPs that were associated with sarcoidosis or complications were extracted from the articles identified. A total of 38 SNPs were found and are listed in Table 1.

**Genotyping:** For participants enrolled from the MEEI Uveitis clinics, two 10 ml vials of whole blood were drawn on each patient at MEEI and frozen at -80 °C for up to several months. Genomic DNA was isolated from whole blood using the Gentra Puregene Blood Kit platform at the Broad Institute (Cambridge, MA). The 38 SNPs were then genotyped on the Sequenom MassARRAY iPLEX 137 platform. HapMap control genotyping was performed to ensure genotype calling accuracy. For participants enrolled from the Partners HealthCare Biobank, whole blood samples were collected as a dedicated research draw or as a clinical discard. Whole blood was spun to buffy coat with a centrifuge and the buffy coat was stored in a freezer for up to several months. The buffy coat was then extracted to DNA using either the Autogen’s Flexstar (Protocol: Isolation of DNA from 100–500 μl Buffy Coat) or Qiagen’s Autopure instruments at the Partners Translational Genomics Core (Boston, MA). The DNA was then stored in an ultralow freezer (-80 °C). DNA was genotyped on the Expanded Multi-Ethnic Genotyping Array at the Partners Translational Genomics Core (Boston, MA). Imputation was performed using the 1000 Genomes reference panel using the Michigan Imputation Server and Minimac3. Genotypes for the 38 SNPs of interest were extracted from this imputed genotype data set and can be found in Appendix 1. Quality control for genotyping quality, missingness, Hardy–Weinberg equilibrium, and imputation quality were performed on the Biobank data using standard protocols. Because the samples for this study were genotyped on two different platforms, we also performed quality control steps on the two data sets (MEEI Uveitis Clinics and Partners HealthCare Biobank) jointly.

**Statistical analysis:** We compared baseline demographic and clinical variables between cases and controls with the t-test for continuous variables and the chi-square test for categorical variables. Any variables that were statistically significantly different between the cases and the controls were adjusted for in the subsequent analyses. All genetic analyses were conducted with the genetic association analysis software, PLINK [26]. Multiple hypotheses testing correction was done with permutation. A corrected p value of less than 0.05 was considered statistically significant. The primary analysis compared all sarcoidosis-associated uveitis patients (cases) to patients with sarcoidosis without uveitis (controls). To eliminate potential bias from differential ancestry, a subanalysis including only Caucasian Americans was performed. Subanalyses were also performed separately in men and women to determine whether there was any gender-specific effect. To further elucidate potential associations between SNPs and particular anatomic subtypes of sarcoidosis-associated uveitis, we performed subanalyses examining sarcoidosis-associated anterior uveitis separately from the other anatomic subtypes: intermediate uveitis, posterior uveitis, and panuveitis. We chose to look at anterior uveitis only in the subanalyses as we lacked sufficient power to examine the other anatomic subtypes of uveitis, as well as retinal vasculitis.

**RESULTS**

A total of 77 participants were enrolled in this study for the primary analysis: 45 patients with sarcoidosis-associated uveitis (cases) and 32 patients with systemic sarcoidosis without uveitis (controls). The clinical and demographic characteristics of all participants are shown in Table 2. Of the 45 patients with sarcoidosis-associated uveitis, 24 patients had anterior uveitis, seven had intermediate uveitis, four had posterior uveitis, and ten had panuveitis. In addition, two patients with posterior uveitis and two patients with panuveitis had evidence of retinal vasculitis. Twenty-eight of the 45 sarcoidosis-related uveitis cases had biopsy-proven sarcoidosis, and 17 participants had presumed sarcoidosis based on the presence of bilateral hilar adenopathy. Of the 32 controls without uveitis, 27 had biopsy-proven sarcoidosis, and five had sarcoidosis diagnosed based on bilateral hilar adenopathy. There were no statistically significant differences in the age, gender, race, or disease duration between the
| Gene Name or Locus | SNP            | Reference                          | Association with                                      |
|-------------------|----------------|------------------------------------|------------------------------------------------------|
| ANXA11            | rs2789679*     | Hofmann et al. 2008 [12]           | Sarcoïdosis                                          |
| ANXA11            | rs2573346*     | Hofmann et al. 2008 [12]           | Sarcoïdosis                                          |
| ANXA11            | rs1049550*     | Hofmann et al. 2008 [12]           | Sarcoïdosis                                          |
| ANXA11            | rs61860052     | Levin et al. 2012 [22]             | Sarcoïdosis in African Americans                     |
| ANXA11 and PLAC9  | rs953600*      | Hofmann et al. 2008 [12]           | Sarcoïdosis                                          |
| BTNL2             | rs9268480*     | Adrianto et al. 2012 [6]           | Sarcoïdosis                                          |
| BTNL2 (upstream)  | rs9268482*     | Adrianto et al. 2012 [6]           | Sarcoïdosis                                          |
| CDCC88B           | rs671976*      | Fischer et al. 2012 [14]           | Sarcoïdosis                                          |
| CDCC88B           | rs479777*      | Fischer et al. 2012 [14]           | Sarcoïdosis                                          |
| CCR 5             | rs1799987      | McDougal et al. 2009 [36]          | Erythema nodosum in sarcoidosis                      |
| CCR 5             | rs333          | Fischer et al. 2008 [37]           | Lofgren syndrome                                     |
| Locus 1q24.3      | rs12035082     | Fischer et al. 2011[38]            | Acute sarcoidosis                                    |
| CFH Y402H         | rs1061170      | Thompson et al. 2013 [18]          | Ocular sarcoidosis                                   |
| C1000RF67/10p12.2 | rs398024*      | Franke et al. 2008 [39]            | Sarcoïdosis                                          |
| HERC2             | rs916977       | Fischer et al. 2011 [38]           | Sarcoïdosis                                          |
| IL23R             | rs11465804 (intron 8) | Kim et al. 2011 [20]                          | Sarcoïdosis, Sarcoïdosis-associated uveitis         |
| IL23R             | rs75117847 (intron 6) | Kim et al. 2011 [20]                          | Sarcoïdosis                                          |
| HSP-70/Hom        | rs2075800      | Spagnolo et al. 2007 [19]          | Sarcoïdosis-associated uveitis                       |
| HSP-70/Hom        | rs403620       | Spagnolo et al. 2007 [19]          | Sarcoïdosis                                          |
| IL23R             | rs1209026      | Fischer et al. 2011 [20, 38]       | Chronic sarcoïdosis                                 |
| IL2RA             | rs2722489†     | Zhou et al. 2012 [40]              | Complicated sarcoïdosis                              |
| LTA               | rs909253       | McDougal et al. 2009 [36]          | Erythema nodosum in sarcoïdosis                      |
| NOTCH4            | rs715290*      | Adrianto et al. 2012 [6]           | Sarcoïdosis                                          |
| OS9               | rs1172300*     | Hofmann et al. 2013 [13]           | Sarcoïdosis                                          |
| OS9               | rs701007*†     | Hofmann et al. 2013 [13]           | Sarcoïdosis                                          |
| OS9               | rs799265*†     | Hofmann et al. 2013 [13]           | Sarcoïdosis                                          |
| RAB23             | rs404061*      | Hofmann et al. 2011 [26]           | Sarcoïdosis                                          |
| TGF-β2            | rs891467       | Kruit et al. 2006 [41]             | Pulmonary fibrosis in sarcoïdosis                    |
| TGF-β3            | rs3917200      | Kruit et al. 2006 [41]             | Pulmonary fibrosis in sarcoïdosis                    |
| TGF-β3            | rs3917165      | Kruit et al. 2006 [41]             | Pulmonary fibrosis in sarcoïdosis                    |
| TLR4              | rs4986790      | Pabst et al. 2006 [16]             | Chronic sarcoïdosis                                 |
| TNF-α             | rs800629       | McDougal et al. 2009 [36]          | Erythema nodosum in sarcoïdosis                      |
| HLA-DQA1          | rsI7843604*    | Adrianto et al. 2012 [6]           | Sarcoïdosis                                          |
| Gene Name or Locus | SNP         | Reference                | Association with |
|-------------------|-------------|--------------------------|------------------|
| HLA- DQB1         | rs149288329*| Adrianto et al. 2012 [6] | Sarcoidosis      |
| HLA- DRA          | rs7192*     | Adrianto et al. 2012 [6] | Sarcoidosis      |
| HLA- DRA          | rs7194*     | Adrianto et al. 2012 [6] | Sarcoidosis      |
| HLA- DRB1         | rs615672*   | Adrianto et al. 2012 [6] | Sarcoidosis      |
| HLA- DRB5         | rs17203612* | Adrianto et al. 2012 [6] | Sarcoidosis      |

*SNPs that were genome-wide significant findings in the previous studies. †Proxy SNPs for originally reported SNP rs1050045
sarcoidosis-related uveitis cases and the controls (all p>0.05). All patients had some degree of pulmonary involvement.

Table 3 shows the association results for the primary analysis that compared cases with sarcoidosis-associated uveitis to controls with sarcoidosis but no uveitis. Three SNPs (rs333, rs149288329, and rs12035082) failed the genotyping quality control parameters and were excluded from the study; thus, the results for 35 SNPs are presented in Appendix 1 and Table 3. SNPs rs1040461 in RAB23 and rs61860052 in ANXA11 on chromosomes 6 and 10, respectively, were associated with sarcoidosis-associated uveitis after p value correction for multiple hypotheses testing (Pcorr). The T allele of rs1040461 and the A allele of rs61860052 were found to occur more frequently in patients with sarcoidosis-related uveitis (Pcorr=0.01 and Pcorr=0.02, respectively).

In this study, 71.4% (55/77) of the patients were Caucasian Americans. We performed a subanalysis in Caucasian Americans only to address potential bias from differential ancestry. The A allele of rs61860052 continued to be associated with uveitis in this subgroup (odds ratio [OR]=4.96, Pcorr=0.01). The T allele of rs1040461 was no longer associated to a statistically significant degree, although the direction of effect was consistent with the finding in the overall group. In addition, two other variants were statistically significantly associated with uveitis in Caucasian Americans: the T allele of rs17203612 (Pcorr=0.009) and the C allele of rs615672 (Pcorr=0.04).

In the subanalysis of women only, the T allele of rs1040461 (Pcorr=0.04) and the T allele of rs17203612 (Pcorr=0.03) were associated with uveitis in sarcoidosis in women after correction for multiple hypothesis testing. No statistically significant associations were identified in the men-only subanalysis.

Table 4 shows the association results for the subanalyses for anterior uveitis. Both of the statistically significantly associated variants in the main analysis (rs1040461 and rs61860052) were also statistically significant in the subanalysis that compared sarcoidosis-associated anterior uveitis versus patients with sarcoidosis without uveitis. The HLA-DRB5 (Gene ID 3127; OMIM 604776) variant that was significant in the Caucasian American– and female-only analyses (rs17203612), was also statistically significant in the anterior uveitis subanalysis; this variant was not statistically significant in the main analysis. Three other variants were also statistically significantly associated in the anterior uveitis subanalyses but not statistically significant in the main analysis: the T allele of rs7192 (Pcorr=0.007), the G allele of rs7194 (Pcorr=0.006), and the A allele of rs1800629 (Pcorr=0.03). When patients with sarcoidosis-associated anterior uveitis were compared to patients with other anatomic subtypes (intermediate, posterior, and panuveitis combined), these three variants remained statistically significantly associated with anterior uveitis. Additionally, the T allele of rs3917165 was statistically significantly associated in the analysis of sarcoidosis-associated anterior uveitis versus other anatomic subtypes of uveitis but not in the analysis of anterior uveitis-only compared to patients with sarcoidosis without uveitis.

For the three previous studies which identified genetic associations with sarcoidosis-associated uveitis specifically [18-20], we extracted data on those SNPs on 102 sarcoidosis-free healthy controls. We compared the sarcoidosis-associated uveitis cases to these healthy controls to mimic the design in

| Patient characteristics | Cases: Sarcoidosis-related uveitis (n = 45)* | Controls: Sarcoidosis without uveitis (n = 32)† | P value |
|-------------------------|---------------------------------------------|-----------------------------------------------|---------|
| Age mean (SD), years    | 58.9 (13.5)                                 | 56.7 (11.1)                                   | 0.44    |
| Gender                  |                                             |                                               |         |
| Male                    | 14                                          | 15                                            | 0.16    |
| Female                  | 31                                          | 17                                            |         |
| Mean duration of sarcoidosis (SD), years | 8.8 (7.4) | 10.0 (10.5) | 0.55 |
| Race                    |                                             |                                               |         |
| CA                      | 31                                          | 25                                            | 0.23    |
| AA                      | 10                                          | 3                                             |         |
| HA                      | 3                                           | 2                                             |         |
| AA                      | 1                                           | 2                                             |         |

SD=standard deviation, CA=Caucasian American, AA=African American, HA=Hispanic American, AA=Asian American *Includes 36 cases recruited from the MEEI Uveitis Clinics and 9 cases recruited from the Partners HealthCare Biobank. † Includes 10 controls recruited from the MEEI Uveitis Clinics and 22 controls recruited from the Partners HealthCare Biobank.
### Table 3. Association results for sarcoidosis-associated uveitis.

| Gene       | Chr | SNP          | Ref/Alternate Allele | Ref Allele Frequency Cases (n=45) | Ref Allele Frequency Controls (n=32) | OR   | 95% CI       | P corr |
|------------|-----|--------------|----------------------|-----------------------------------|--------------------------------------|------|--------------|--------|
| CFH Y402H  | 1   | rs1061170    | C/T                  | 0.34                              | 0.37                                 | 0.87 | 0.45–1.71    | 0.85   |
| IL23R      | 1   | rs11209026   | A/G                  | 0.07                              | 0.04                                 | 1.52 | 0.36–6.34    | 0.48   |
| IL23R      | 1   | rs1446804    | G/T                  | 0.07                              | 0.06                                 | 1.07 | 0.29–3.96    | 0.85   |
| IL23R      | 1   | rs7517847    | G/T                  | 0.38                              | 0.29                                 | 1.51 | 0.48–4.78    | 0.47   |
| TGF-β2     | 1   | rs1891467    | G/A                  | 0.31                              | 0.23                                 | 1.50 | 0.72–3.12    | 0.34   |
| CCR 5      | 3   | rs1799987    | A/G                  | 0.51                              | 0.45                                 | 1.26 | 0.66–2.40    | 0.85   |
| BTN1L2     | 6   | rs9268480    | T/C                  | 0.31                              | 0.22                                 | 1.63 | 0.77–3.45    | 0.17   |
| BTN1L2     | 6   | rs9268482    | T/A                  | 0.31                              | 0.22                                 | 1.63 | 0.77–3.45    | 0.17   |
| HLA- DQA1  | 6   | rs17843604   | T/C                  | 0.49                              | 0.48                                 | 1.02 | 0.54–1.93    | 1.00   |
| HLA- DRA   | 6   | rs7192       | T/G                  | 0.35                              | 0.47                                 | 0.61 | 0.32–1.18    | 0.17   |
| HLA- DRA   | 6   | rs7194       | G/A                  | 0.35                              | 0.47                                 | 0.61 | 0.32–1.18    | 0.17   |
| HLA- DRB1  | 6   | rs615672     | C/G                  | 0.40                              | 0.48                                 | 0.71 | 0.37–1.35    | 0.34   |
| HLA- DRB5  | 6   | rs17203612   | T/A                  | 0.41                              | 0.25                                 | 2.06 | 1.01–4.19    | 0.07   |
| HSP-70/Hom | 6   | rs2075800    | T/C                  | 0.30                              | 0.35                                 | 0.76 | 0.38–1.51    | 0.50   |
| HSP-70/Hom | 6   | rs1043620    | T/C                  | 0.03                              | 0.08                                 | 0.31 | 0.05–1.65    | 0.24   |
| LTA        | 6   | rs909255     | G/A                  | 0.50                              | 0.36                                 | 1.78 | 0.92–3.44    | 0.15   |
| NOTCH4     | 6   | rs715299     | G/T                  | 0.38                              | 0.31                                 | 1.37 | 0.69–2.71    | 0.40   |
| RAB23      | 6   | rs1040461    | T/C                  | 0.02                              | 0.00*                                | N/A  | N/A          | 0.01   |
| TNF-α      | 6   | rs1800629    | A/G                  | 0.16                              | 0.17                                 | 0.94 | 0.39–2.23    | 1.00   |
| TLR4       | 9    | rs4986790    | G/A                  | 0.02                              | 0.07                                 | 0.31 | 0.05–1.43    | 0.10   |
| ANXA11     | 10  | rs2789679    | T/A                  | 0.31                              | 0.41                                 | 0.66 | 0.34–1.29    | 0.18   |
| ANXA11     | 10  | rs2573346    | A/G                  | 0.33                              | 0.41                                 | 0.71 | 0.36–1.38    | 0.27   |
| ANXA11     | 10  | rs1049550    | A/G                  | 0.34                              | 0.41                                 | 0.74 | 0.38–1.45    | 0.35   |
| ANXA11     | 10  | rs61860052   | A/C                  | 0.13                              | 0.03                                 | 4.77 | 1.03–22.11   | 0.02   |
| ANXA11 and PLAC9 | 10  | rs1953600 | T/C                  | 0.32                              | 0.41                                 | 0.71 | 0.36–1.38    | 0.26   |
| C10orf67/10p12.2 | 10  | rs1398024 | T/G                  | 0.21                              | 0.20                                 | 1.05 | 0.47–2.32    | 1.00   |
| IL2RA      | 10  | rs12722489   | T/C                  | 0.13                              | 0.08                                 | 1.73 | 0.57–5.26    | 0.31   |
| CDCC888B   | 11  | rs671976     | G/A                  | 0.50                              | 0.50                                 | 1.00 | 0.52–1.91    | 1.00   |
| CDCC888B   | 11  | rs479777    | C/T                  | 0.26                              | 0.28                                 | 0.88 | 0.42–1.82    | 0.86   |
| OS9        | 12   | rs11172300   | T/C                  | 0.36                              | 0.36                                 | 1.00 | 0.51–1.97    | 0.86   |
| OS9        | 12   | rs701007     | A/C                  | 0.40                              | 0.42                                 | 0.91 | 0.48–1.75    | 0.86   |
| Gene | Chr | SNP      | Ref/Alternate Allele | Ref Allele Frequency Cases (n=45) | Ref Allele Frequency Controls (n=32) | OR   | 95% CI       | P corr |
|------|-----|----------|----------------------|-----------------------------------|-------------------------------------|------|--------------|--------|
| OS9  | 12  | rs799265 | G/A                  | 0.43                              | 0.42                                | 1.05 | 0.55–2.00    | 1.00   |
| TGF-β3 | 14 | rs3917200 | G/A                  | 0.17                              | 0.09                                | 2.04 | 0.74–5.60    | 0.31   |
| TGF-β3 | 14 | rs3917165 | T/C                  | 0.12                              | 0.08                                | 1.55 | 0.50–4.79    | 0.59   |
| HERC2 | 15  | rs916977 | T/C                  | 0.33                              | 0.30                                | 1.14 | 0.57–2.42    | 0.85   |

*Not present in any controls. Chr=chromosome, CI=confidence interval, SNP=single nucleotide polymorphism, OR=odds ratio, CI=confidence interval, Pcorr=P value corrected for multiple testing by permutation, Ref Allele=reference allele, N/A=not available
Table 4. Association results for sarcoidosis-associated anterior uveitis.

| Gene   | Chr | SNP      | Ref/Alternate Allele | Ref Allele Freq, Cases (n=24) | Ref Allele Freq, Controls (n=32) | OR  | 95% CI     | Pcorr |
|--------|-----|----------|----------------------|-------------------------------|---------------------------------|-----|-----------|-------|
| CFH Y402H | 1   | rs1061170 | C/T                 | 0.33                          | 0.38                            | 0.83 | 0.38–1.83 | 1.00  |
| IL23R  | 1   | rs11209026| A/G                 | 0.09                          | 0.05                            | 2.03 | 0.43–9.57 | 0.29  |
| IL23R  | 1   | rs11465804| G/T                 | 0.08                          | 0.06                            | 1.36 | 0.32–5.75 | 0.73  |
| IL23R  | 1   | rs7517847 | G/T                 | 0.39                          | 0.30                            | 1.52 | 0.48–4.78 | 0.50  |
| TGF-β2 | 1   | rs1891467 | G/A                 | 0.39                          | 0.23                            | 2.06 | 0.89–4.76 | 0.09  |
| CCR 5  | 3   | rs1799987 | A/G*                | 0.52                          | 0.45                            | 1.31 | 0.62–2.78 | 0.50  |
| BTN2L  | 6   | rs9268480 | T/C                 | 0.34                          | 0.22                            | 1.85 | 0.78–4.37 | 0.14  |
| HLA- DQA1| 6  | rs17843604| C/T*                | 0.46                          | 0.52                            | 0.79 | 0.38–1.68 | 0.45  |
| HLA- DRA| 6   | rs7192    | T/G                 | 0.23                          | 0.47                            | 0.33 | 0.14–0.79 | 0.007 |
| HLA- DRA| 6   | rs7194    | G/A                 | 0.23                          | 0.47                            | 0.33 | 0.14–0.79 | 0.006 |
| HLA- DRB1| 6  | rs615672  | C/G                 | 0.35                          | 0.48                            | 0.58 | 0.27–1.26 | 0.34  |
| HLA- DRB5| 6  | rs17203612| T/A                 | 0.50                          | 0.25                            | 3.00 | 1.32–6.80 | 0.014 |
| HSP-70/Hom | 6 | rs2075800 | T/C                 | 0.35                          | 0.36                            | 0.98 | 0.45–2.14 | 1.00  |
| HSP-70/Hom | 6 | rs1043620 | T/C                 | 0.02                          | 0.08                            | 0.27 | 0.03–2.35 | 0.26  |
| LTA    | 6   | rs909253  | G/A                 | 0.42                          | 0.36                            | 1.27 | 0.59–2.75 | 0.64  |
| NOTCH4 | 6   | rs715299  | G/T                 | 0.45                          | 0.31                            | 1.83 | 0.83–4.06 | 0.20  |
| RAB23  | 6   | rs1040461 | T/C                 | 0.11                          | 0.00                            | N/A  | N/A       | 0.001 |
| TFG-α  | 6   | rs1800629 | A/G                 | 0.05                          | 0.17                            | 0.23 | 0.05–1.09 | 0.03  |
| TLR4   | 9    | rs4987990 | G/A                 | 0.02                          | 0.08                            | 0.25 | 0.03–2.22 | 0.20  |
| ANXA11 | 10  | rs2789769 | T/A                 | 0.33                          | 0.41                            | 0.73 | 0.33–1.60 | 0.34  |
| ANXA11 | 10  | rs2537346 | A/G                 | 0.36                          | 0.41                            | 0.84 | 0.38–1.84 | 0.55  |
| ANXA11 | 10  | rs1049550 | A/G                 | 0.39                          | 0.41                            | 0.92 | 0.42–2.02 | 0.64  |
| ANXA11 | 10  | rs61860052| A/C                 | 0.19                          | 0.03                            | 7.15 | 1.47–34.86 | 0.004 |
| ANXA11 and PLAC9 | 10 | rs1953600 | T/C                 | 0.36                          | 0.41                            | 0.84 | 0.38–1.84 | 0.55  |
| C100R67/10p12.2 | 10 | rs1398024 | T/G                 | 0.23                          | 0.20                            | 1.17 | 0.47–2.89 | 0.86  |
| IL2RA  | 10  | rs12722489| T/C                 | 0.16                          | 0.08                            | 2.23 | 0.66–7.55 | 0.18  |
| Gene     | Chr | SNP         | Ref/Alternate Allele | Anterior Uveitis versus Controls | Anterior Uveitis versus Other (Intermediate, Posterior and Pan) Uveitis |
|----------|-----|-------------|----------------------|----------------------------------|-------------------------------------------------|
|          |     |             | Ref Allele Freq, Cases (n=24) | Ref Allele Freq, Controls (n=32) | OR | 95% CI | P corr | Ref Allele Freq, Ant Uveitis Cases (n=24) | Ref Allele Freq, Other Uveitis (n=21) | OR | 95% (CI) | P corr |
| CDCC88B  | 11  | rs671976    | A/G                  | 0.41                            | 0.50 | 0.69 | 0.32–1.50 | 0.32 | 0.59 | 0.40 | 2.12 | 0.90–5.02 | 0.11 |
| CDCC88B  | 11  | rs479777    | C/T                  | 0.23                            | 0.28 | 0.75 | 0.31–1.83 | 0.63 | 0.23 | 0.29 | 0.74 | 0.28–1.94 | 0.69 |
| OS9      | 12  | rs11172300  | T/C                  | 0.34                            | 0.36 | 0.92 | 0.41–2.06 | 0.69 | 0.34 | 0.38 | 0.84 | 0.35–2.03 | 0.64 |
| OS9      | 12  | rs701007    | A/C                  | 0.38                            | 0.42 | 0.82 | 0.38–1.77 | 0.44 | 0.38 | 0.43 | 0.80 | 0.34–1.86 | 0.48 |
| OS9      | 12  | rs799265    | G/A                  | 0.40                            | 0.42 | 0.90 | 0.42–1.92 | 0.78 | 0.40 | 0.48 | 0.72 | 0.31–1.67 | 0.38 |
| TGF-β3   | 14  | rs3917200   | G/A                  | 0.23                            | 0.09 | 2.84 | 0.95–8.52 | 0.07 | 0.23 | 0.12 | 2.18 | 0.68–7.01 | 0.20 |
| TGF-β3   | 14  | rs3917165   | T/C                  | 0.18                            | 0.08 | 2.62 | 0.80–8.64 | 0.07 | 0.18 | 0.05 | 4.44 | 0.89–22.31 | 0.03 |
| HERC2    | 15  | rs916977    | T/C                  | 0.32                            | 0.30 | 1.11 | 0.48–2.54 | 1.00 | 0.32 | 0.33 | 0.93 | 0.38–2.30 | 0.86 |

*Effect and reference allele are reversed for anterior uveitis versus other uveitis*
the previous studies. We were not able to replicate any of the findings to a statistically significant degree.

**DISCUSSION**

In this study, four variants that had been previously associated with overall sarcoidosis were found to be associated with sarcoidosis-associated uveitis in the entire cohort or in a subpopulation. The most consistent evidence for association was with SNP rs1040461. It was associated with sarcoidosis-associated uveitis in the overall population, as well as in the women-only and sarcoidosis-associated anterior uveitis versus non-ocular sarcoidosis subanalysis, and the effect was in the same direction as that seen originally for overall sarcoidosis. This SNP was initially identified as a novel susceptibility locus for sarcoidosis in 2011 as part of a genome-wide association study; however, the researchers did not evaluate ocular involvement in that study [27]. In that study, the T allele increased the risk of developing sarcoidosis (OR = 1.68, Pcorr = 0.01). This association was replicated in an independent case–control sample, and quantitative mRNA expression studies pointed to the RAB23 gene as the most likely to be implicated in pathogenesis by this variant [27]. In the current study, this association is the same direction with the T allele increasing the risk of developing sarcoidosis-associated uveitis. RAB23 is proposed to be involved in bactericidal defense and regulation of the sonic hedgehog signaling pathway [28]. Many reports describe the presence of microbial cell wall agents in tissues of patients with sarcoidosis, and several clinical studies have demonstrated the occurrence of microbes in patients with sarcoidosis [29,30].

This association of rs1040461 did not reach statistical significance in the Caucasian American subanalysis (Pcorr=0.10). This may have been due to reduced power in this subanalysis. Although the association in this subanalysis did not reach statistical significance, the direction of effect was consistent with the direction in the overall group with the T allele being more frequent in cases. The T allele frequency was 5% in the Caucasian American cases versus 0% of the Caucasian American controls. The direction of effect was also consistent in African Americans where the T allele frequency was 10% in the African American cases versus 0% in the African American controls.

The other allele that was statistically significantly associated with sarcoidosis-associated uveitis in the primary analysis was the A allele of rs61860052. The SNP rs61860052 in ANXA11, which encodes the annexin A11 protein, was associated with sarcoidosis initially in an African American population (p=0.01) in 2013 [22]. In that study the frequencies of the A allele were 1% and 2% in their cases and controls, respectively. In the current study, the frequency of the A allele was 13.5% in the sarcoidosis uveitis cases versus 3% in the controls overall. Thus, the A allele, which was associated with lower risk of overall sarcoidosis in the initial African American study, was associated with a higher risk of sarcoidosis-associated uveitis in the current study. When we examined the allele frequencies in Caucasian Americans and African Americans separately in this study, the A allele was increased in sarcoidosis-associated uveitis in both populations: The A allele frequency was 5% in the African American cases versus 0% in the African American controls and 17.7% in the Caucasian American cases versus 4% in the Caucasian American controls. For comparison, the population frequencies of this allele in the 1000 Genomes database are 10% in Europeans and 0.3% in Africans, respectively. The association of this allele also achieved significance in the Caucasian American–only analysis and the sarcoidosis-associated anterior uveitis versus non-ocular sarcoidosis subanalysis.

The reason why the allele in this gene, which decreases risk of overall sarcoidosis, would increase the risk of sarcoidosis-associated uveitis is unclear. It is possible this variant confers a specific risk to the phenotype of sarcoidosis-associated uveitis. An alternative explanation for this finding is that because of the relatively low allele frequency, particularly with African ancestry, there is instability in the present results, and they represent an artifact rather than a true association. Annexins have been previously implicated in autoimmune disorders, and annexin A11 is among the few proteins that are detectable in human B-cell exosomes [6,22,31,32]. Exosomes are thought to induce immune responses or tolerance depending on their cellular origin. Their role in the activation of B-cells in sarcoidosis has been proposed, and this activation could lower the threshold for T-cell activation [33,34]. Decreased activation of CD8+ and CD19+ immune cells is a proposed mechanism for sarcoidosis [12].

Two additional variants were associated with uveitis only in the Caucasian Americans subanalysis: rs17203612 and rs615672. These variants were originally identified as novel susceptibility loci for sarcoidosis in genome-wide association studies [6,12]. Both are within major histocompatibility complex (MHC) genes on chromosome 6, in HLA-DRB5 and HLA-DRB1, respectively. The MHC class II region is known for its major role in immune-mediated disorders [35]. Association of HLA-DRBI with uveitis has also been identified in juvenile idiopathic arthritis, and the HLA-DRB1*1301 allele is associated with ANA positivity [36]. In addition to being significant in the Caucasian Americans subanalysis, the T allele at rs17203612 was associated with sarcoidosis-associated
uveitis in the women-only and anterior uveitis–only versus patients with sarcoidosis without uveitis subanalyses. In the original study, the T allele of rs17203612 increased the risk of sarcoidosis in African Americans (OR=1.56, Pcorr=2.66 × 10−5) and in Caucasian Americans (OR=1.58, Pcorr=1.82 × 10−5) [6]. In the current study, the T allele frequency of rs17203613 was 53% in the Caucasian American cases with sarcoidosis-associated uveitis versus 28% in the Caucasian American controls with sarcoidosis but without uveitis. In the women-only analysis, the allele frequency was 41% in cases versus 17.5% in controls. The direction of effect was also consistent in the men-only analysis (allele frequencies of 40% in cases versus 33% in controls) although it did not reach statistical significance perhaps because of the limited sample size. Therefore, the same allele that increased risk of sarcoidosis overall in the original study also increases risk of sarcoidosis-associated uveitis in the current study. The direction of effect was also the same for the primary analysis that included all ancestry groups but was shy of achieving statistical significance.

The G allele of rs615672 was associated with an increased risk of uveitis in the Caucasian American–only analysis. The original association between the G allele of rs615672 and sarcoidosis was found in African Americans (OR=0.68, p=7.9 × 10−5) and in Caucasian Americans (OR=0.81, p=0.008) [6]. In the present study, the G allele frequency of rs615672 was 71% in cases versus 52% in controls among Caucasian Americans and 25% in cases versus 16.7% in controls among African Americans. Therefore, similar to the results for rs61860052, the allele that decreased risk for sarcoidosis in the original study increased risk of sarcoidosis-associated uveitis in the current study. We cannot rule out that this might be a false positive finding because of the small sample in this study. The population frequencies of this allele in the 1000 Genomes database are 78% in Europeans and 51% in Africans.

The subanalyses examining whether particular genotypes could be correlated to the specific anterior subtype of sarcoidosis-associated uveitis revealed that the two SNPs identified in the primary analysis (rs1040461 and rs61860052) were statistically significant in the analysis where only patients with sarcoidosis-associated anterior uveitis were cases. Three variants were statistically significantly associated with anterior uveitis but were not statistically significantly associated with uveitis in the primary analysis: rs7192, rs7194, and rs1800629. These variants were statistically significant when anterior uveitis cases were compared to patients with sarcoidosis without uveitis and when anterior uveitis cases were compared to patients with intermediate uveitis, posterior uveitis, and panuveitis. Additionally, the T allele of rs3917165 was statistically significantly associated in the analysis of sarcoidosis-associated anterior uveitis versus other anatomic subtypes of uveitis but not in the main analysis or the analysis of anterior uveitis–only compared with patients with sarcoidosis without uveitis. These results indicate that these variants could specifically modify risk for anterior uveitis, but further investigation in larger data sets is necessary.

Variants rs7192 and rs7194 are located in the HLA-DRA (Gene ID 3122; OMIM 142860) gene which encodes the alpha subunit of the HLA-DR loci and assists with the presentation of peptides derived from extracellular proteins. In a previous study, HLA-DRA was shown to be associated with uveitis, though this study did not test specifically for anterior uveitis [37]. Variant rs1800629 is located in the promoter of the TNF-α gene (Gene ID 7124; OMIM 191160). The A allele of this SNP, which had an allele frequency of 5% of patients with sarcoidosis-associated anterior uveitis, 17% of patients with sarcoidosis without uveitis, and 29% of patients with other anatomic subtypes of sarcoidosis-associated uveitis other than anterior uveitis, is associated with increased expression of TNF-α. One interpretation of these data is that increased TNF-α expression may specifically associated with increased risk of intermediate uveitis, posterior uveitis, and panuveitis, which are typically more severe and visually threatening than anterior uveitis. This SNP, particularly the A allele, is associated with several autoimmune diseases, such as ankylosing spondylitis, rheumatoid arthritis, and systemic lupus erythematosus [38]. Variant rs3917165 is located in the TGFβ3 gene (Gene ID 7043; OMIM 190230) which encodes the transforming growth factor beta-3 protein: a cytokine involved in cell differentiation, embryogenesis, and possibly wound healing. TGFβ3 has been previously described to be associated with uveitis, but specific subtypes have not been examined [39].

The present results differ from those found in previous genetic studies of sarcoidosis-associated uveitis. One important difference between the present study and most of these previous studies is that the controls in the present study were patients with sarcoidosis and no uveitis, as opposed to healthy controls without sarcoidosis. The primary analysis case–control definition was designed specifically to identify genes that convey risk to uveitis, whereas the previous study’s case–control definition could also have been detecting risk alleles for sarcoidosis overall. For example, Thompson et al. performed a study comparing 41 subjects with ocular sarcoidosis to 393 sarcoidosis-free controls [18]. They found that the C allele at rs1061170 in CFH was present at a higher frequency
in the cases versus controls (48.7% versus 35%, OR=1.72, p=0.018). When we used the same case–control definition, we also found that the C allele was more frequent in cases than controls, although it did not reach statistical significance (OR=1.34, p=0.28). However, in the primary analysis case–control definition, the C allele was slightly more common in the controls (34.4% in cases, 37.5% in controls, OR=0.88, p=0.77). Therefore, it is possible that this variant is associated with sarcoidosis overall, rather than sarcoidosis-associated uveitis specifically.

Similarly, Kim et al. performed a study comparing 91 subjects with sarcoidosis including 58 with ocular sarcoidosis to 104 sarcoidosis-free controls [20]. They showed among 11 IL-23R SNPs, two of the SNPs, rs11465804 and rs11209026, were associated with the uveitis subgroup compared to healthy controls without sarcoidosis. We did not find any association with these two SNPs in the primary analysis, and the direction of effect observed was not consistent with the original study. When we attempted to replicate the findings from this study more precisely using a control definition that matched theirs of sarcoidosis-free healthy controls, we still were not able to replicate the results despite having similar power: The G allele of rs11465804 was present in 6.7% of cases and 8.9% of healthy controls (OR=0.73 and p=0.52), and the A allele of rs11209026 was present in 7.0% of cases and 7.4% of healthy controls (OR=0.94 and p=0.89).

The third previous study that looked for genetic associations for sarcoidosis-associated uveitis specifically was conducted by Spagnolo et al. They evaluated five SNPs in 270 Caucasian American patients with sarcoidosis, including 88 with sarcoid-related uveitis, and in 347 matched healthy control subjects [19]. They reported that the HSP-70/Hom rs2075800 G allele was associated with sarcoidosis-related uveitis. The HSP-70/Hom rs2075800 G allele frequency was higher in the sarcoid-uveitis group than in the sarcoid non-uveitis and healthy control groups. We did not find a statistically significant difference for this allele in the primary analysis, although the direction of effect was the same as that seen in the Spagnolo et al. study. One reason that this association may not have reached statistical significance is the more limited power in the current study.

Few genetic studies specifically examined the commonly encountered phenotypic variant of ocular involvement in sarcoidosis. The strengths of the present study include well-characterized case and control definitions to specifically detect an association with the uveitis complication among patients with sarcoidosis. We also examined the largest number of variants for this phenotype to date. All 38 SNPs chosen had a strong a priori hypothesis for being associated with sarcoidosis-associated uveitis. Previously, the largest number of SNPs examined for this phenotype was five [19].

However, the present study has several limitations. First, we had a limited sample size, and larger samples are needed to confirm these initial findings. We performed simulations to characterize the power of this study for a range of allele frequencies and effect sizes using the Genetic Power Calculator. Although we had greater than 80% power to detect genetic variants of large effect sizes, as that seen with rs61860052, we had limited power to detect associations with variants of modest effect. For example, we had only 30% power to detect a variant with a risk allele frequency of 30% and an OR of 1.5. Therefore, it is possible that some of the other variants examined in this study might be associated with ocular sarcoidosis, but larger sample sizes would be required to detect the association. Additionally, we were unable to examine certain subtypes of sarcoidosis-associated uveitis (retinal vasculitis and intermediate uveitis, posterior uveitis, and panuveitis) because of the limited sample size.

We included participants of different ethnicities to maximize the power of the analyses, but this diversity has the potential to introduce population stratification and lead to false positive findings. We mitigated this problem by performing a subanalysis of Caucasian American participants only and by examining allele frequencies in Caucasian Americans and African Americans separately and did not find any overt evidence of population stratification. We genotyped participants on two different platforms which can also lead to false positive findings. To mitigate this problem, we performed strict quality control of genotyping in both data sets and examined the genotyping results carefully across the two platforms, particularly for the significant findings.

There were also limitations regarding clinical characterization of the patients. Approximately half of the participants had presumed sarcoidosis based on bilateral hilar adenopathy but did not have biopsy-proven disease. However, most large GWASs and candidate gene studies have included patients with sarcoidosis who did not have biopsy-proven sarcoidosis as long as they have bilateral hilar adenopathy and other supportive clinical findings according to established criteria [6,14,22,23]. Although the duration of sarcoidosis was slightly longer in the controls than in the cases, there is still the potential for misclassification bias; for example, some of the controls may develop eye involvement with longer follow-up. Misclassification, however, should bias the results to the null so this is unlikely to change the findings that were statistically significant. It is also possible that the associations we detected are associations with more severe sarcoidosis rather than uveitis specifically. However, we did
not find any statistically significant differences between the cases and controls for other SNPs previously associated with sarcoidosis severity specifically.

In conclusion, we identified polymorphisms that were previously associated with increased risk of overall sarcoidosis to also be associated with an increased risk of uveitis in sarcoidosis. The findings of this study will need to be replicated in larger, independent patient populations. If the findings are confirmed, fine-mapping of these genetic loci may yield insights into the underlying pathogenesis of ocular involvement in sarcoidosis and help identify potential treatment targets.

APPENDIX 1. GENOTYPE DATA FOR SNPS ASSOCIATED WITH SARCOIDOSIS-ASSOCIATED UVEITIS (CASES) VS. SARCOIDOSIS PATIENTS WITHOUT UVEITIS (CONTROLS).

To access the data, click or select the words “Appendix 1”

ACKNOWLEDGMENTS

Funding for this study was provided by a research grant from the Massachusetts Lions Eye Research Fund. We wish to thank Partners HealthCare Biobank for providing samples, genomic data, and health information data for a part of our study.

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