Published in final edited form as:
Hum Genet. 2013 July ; 132(7): 803–810. doi:10.1007/s00439-013-1292-5.

Replication of genetic loci for sarcoidosis in US black women: data from the Black Women's Health Study

Yvette Cozier,
Slone Epidemiology Center, Boston University, Boston, USA

Edward Ruiz-Narvaez,
Slone Epidemiology Center, Boston University, Boston, USA

Craig McKinnon,
Slone Epidemiology Center, Boston University, Boston, USA

Jeffrey Berman,
The Pulmonary Center, Boston University, Boston, USA

Lynn Rosenberg, and
Slone Epidemiology Center, Boston University, Boston, USA

Julie Palmer
Slone Epidemiology Center, Boston University, Boston, USA

Yvette Cozier: yvettec@bu.edu

Abstract

In the United States, incidence and mortality from sarcoidosis, a chronic, granulomatous disease, are increased in black women. In data from the Black Women's Health Study, a follow-up of US black women, we assessed two SNPs (rs2076530 and rs9268480) previously identified in the *BTNL2* gene (chromosome 6p21), of which rs4424066 and rs3817963 are perfect proxies, to determine if they represent independent signals of disease risk. We also assessed whether local ancestry in four genomic regions previously identified through admixture mapping was associated with sarcoidosis. Finally, we assessed the relation of global percent African ancestry to risk. We conducted a nested case–control study of 486 sarcoidosis cases and 943 age- and geography-matched controls. Both *BTNL2* SNPs were associated with risk of sarcoidosis in separate models, but in a combined analysis the increased risk was due to the A-allele of the rs3817963 SNP; each copy of the A-allele was associated with a 40 % increase in risk of sarcoidosis ($p = 0.02$) and was confirmed by our haplotypic analysis. Local African ancestry around the rs30533 ancestry informative marker at chromosome 5q31 was associated with a 29 % risk reduction ($p = 0.01$). Therefore, we adjusted our analysis of global African ancestry for number of copies of African alleles in rs30533. Subjects in the highest quintile of percent African ancestry had a 54 % increased risk of sarcoidosis. The present results from a population of African-American women support the role of the *BTNL2* gene and the 5q31 locus in the etiology of sarcoidosis, and also demonstrate that percent African ancestry is associated with disease risk.

Correspondence to: Yvette Cozier, yvettec@bu.edu.

Conflict of interest The authors declare no conflict of interest.

Introduction

In the United States, incidence and mortality from sarcoidosis, a chronic, granulomatous disease, are highest in black women (Iannuzzi et al. 2007; Rybicki et al. 1998; Swigris et al. 2011). There is evidence that common genetic variants influence risk. A genome-wide linkage analysis in 63 German families reported linkage of sarcoidosis to the MHC region in chromosome 6p21 (Schurmann et al. 2001), while a family-based association scan of 244 African-American families found an association between DQCAR, a genetic marker in the MHC region, and sarcoidosis (Rybicki et al. 2003). A subsequent genome-wide linkage sib-pair analysis in 229 African-American families reported linkage peaks at several chromosomes but failed to detect linkage at the MHC region (Iannuzzi et al. 2005). Deeper scanning of the MHC region in white German subjects by Valentonyte and colleagues identified a single nucleotide polymorphism (SNP) in the butyrophilin-like protein 2 (*BTNL2*) gene on chromosome 6p21 (rs2076530) associated with sarcoidosis (Valentonyte et al. 2005). A study of African-American subjects failed to replicate the association between rs2076530 and sarcoidosis (Rybicki et al. 2005b). However, the haplotype that combines rs2076530 with a nearby SNP (rs9268480) was associated with sarcoidosis in both black and white subjects (Rybicki et al. 2005b). Associations of rs2076530 with sarcoidosis risk have also been observed in a study of familial sarcoidosis (Coudurier et al. 2009) and in a case–control study in Denmark (Milman et al. 2011).

In a separate effort, Rybicki and colleagues conducted a genome-wide ancestry mapping scan using over 1,300 ancestry informative markers (AIMs) in African-Americans to determine whether ancestry-linked genes were associated with susceptibility to sarcoidosis, disease severity, or both (Rybicki et al. 2011). Significant associations were observed for several genomic regions, including rs35397 at 5p13, rs11966463 at 6p22, rs30533 at 5q31, and rs1462906 at 8p12 (Rybicki et al. 2011).

Based on data from the Black Women's Health Study (BWHS) (Cozier et al. 2011), a follow-up of US black women, we sought to determine whether the SNPs identified in *BTNL2* represent independent signals of risk of sarcoidosis in African-Americans. We also sought to replicate associations of four genomic regions previously identified through admixture mapping with sarcoidosis risk, and to determine whether global percent African ancestry is related to risk.

Materials and methods

Study population

The human subjects' protocol for this study was approved by the Boston University Medical Center Institutional Review Board. We conducted a nested case–control study within the BWHS. The BWHS began in 1995 when 59,000 women aged 21–69 years enrolled through postal health questionnaires. The members of the cohort self-identified as “black”. Follow-up questionnaires are sent every 2 years. Follow-up of the baseline cohort through the completed 2-year cycles to date has averaged greater than 80 %.

Ascertainment and validation of the diagnosis of sarcoidosis

On the 1995 baseline questionnaire, BWHS participants provided data on demographic and lifestyle factors and medical history. Participants were asked if a physician had ever told them that they had any of a list of medical conditions. The list of diagnoses did not specify sarcoidosis, but women could write it under “other conditions”. The 1997 and all subsequent follow-up questionnaires asked specifically about sarcoidosis.

Women who reported a diagnosis of sarcoidosis were asked for permission to contact their physicians for information on diagnosis and treatment (Cozier et al. 2011). The physicians were asked to complete an assessment questionnaire with questions about the participant's diagnosis and treatment or to provide a copy of the patient's medical records pertaining to sarcoidosis. To date, the diagnosis of sarcoidosis has been confirmed for 96 % of the 148 cases for whom physician questionnaires or medical records were obtained. Among the six disconfirmed cases, one had asthma and one had keloids; four had no diagnostic evidence of sarcoidosis, but the physician did not provide an alternative diagnosis. Based on the high level of agreement between self-report and physician report/records, all women who reported incident sarcoidosis on a BWHS questionnaire were included as cases of sarcoidosis unless the diagnosis was disconfirmed by medical data.

Assessment of disease severity

A supplemental sarcoidosis questionnaire sent to both incident and prevalent cases asked if they had experienced any of a list of nine symptoms at the time of their diagnosis (e.g., shortness of breath, cough, fatigue) (Cozier et al. 2011). The 2011 BWHS follow-up questionnaire included questions assessing current general health and physical function, and levels of fatigue and pain within the past 7 days, based on a previously developed and validated instrument (Cella et al. 2010). Subjects were asked “In general, would you say your health is…” (Excellent, very good, good, fair, poor); “To what extent are you able to carry out your everyday physical activities such as walking, climbing stairs, carrying groceries, or moving a chair?” (completely, mostly, moderately, a little, not at all); “In the past 7 days, how would you rate your fatigue on average?” (none, mild, moderate, severe, very severe); and, “In the past 7 days, how would you rate your pain on average?” (0 = no pain through 10 = worst pain). These responses were used to assess the association of the *BTNL2* SNPs and AIMs and risk of saroidoisis among cases classified according to disease severity.

Collection of DNA samples

DNA samples were obtained from BWHS participants by the mouthwash-swish method (Cozier et al. 2004), with all samples stored in freezers at −80 °C. Approximately, 50 % of participants, 26,800 women, provided a mouthwash-saliva sample. Women who provided samples were slightly older than women who did not, but the two groups were similar with regard to educational level, geographic region of residence, and body mass index.

Cases and controls for the present study

The present study includes 486 cases of sarcoidosis that provided a DNA sample and were diagnosed through the end of the 2009 follow-up cycle; 291 were prevalent cases at baseline and 195 were incident cases that occurred during follow-up. We selected up to two matched controls per case among BWHS participants who had provided a DNA sample and were free of sarcoidosis at the end of the 2009 follow-up period, for a total of 943 controls. Controls were matched to cases on year of birth (±1 year) and geographical region of residence (Northeast, South, Mid-west, and West).

SNP selection

BTNL2 gene—Because we were unable to design primer and probe combinations for rs2076530 and rs9268480, we selected two SNPs that are in complete LD ($r^2 = 1.0$) with the candidate SNPs: rs4424066 (perfect proxy of rs2076530 in HapMap YRI, and $r^2 = 0.97$ in HapMap African ancestry in Southwest USA (ASW)) and rs3817963 (perfect proxy of rs9268480 in HapMap YRI, and $r^2 = 0.95$ in HapMap ASW).

Ancestry informative markers—We also selected 30 ancestral informative markers (AIMs) to estimate the percent African (vs. European) ancestry among participants and to control for population stratification due to differences in admixture. These 30 AIMs are the top SNPs of the 1,500 + "phase 3" admixture panel (Reich et al. 2005). We have previously shown that ancestry estimates based on the set of 30 AIMs are highly correlated with estimates derived from the entire phase3 admixture panel ($r = 0.89$, $p < 0.0001$) (Ruiz-Narvaez et al. 2011). We included an additional four AIMs that were reported in an admixture mapping study to be associated with sarcoidosis in African-Americans: rs35397 (chromosome 5p13), rs11966463 (chromosome 6p22), rs30533 (chromosome 5q31), and rs1462906 (chromosome 8p12) (Rybicki et al. 2011).

Genotyping and quality control—DNA was isolated from the mouthwash-saliva samples by use of the QIAAMP DNA Mini Kit (Qiagen, Valencia, CA, USA, http://www.quiagen.com). Whole genome amplification was performed with the Qiagen RePLI-g Kits using the method of multiple displacement amplification. Amplified samples underwent purification and Pico Green quantification at the Broad Institute Center for Genotyping and Analysis (Cambridge, MA) before being plated for genotyping.

Genotyping was carried out at the Broad Institute Center for Genotyping and Analysis using the Sequenom Mass-Array iPLEX technology. Along with the cases and controls, 32 blinded duplicate samples were included to assess reproducibility of the genotypes. An average reproducibility of 98.5 % was obtained among the blinded duplicates. All SNPs with calling rate <90 % or a deviation from Hardy–Weinberg equilibrium in the control samples at $p < 0.001$ were excluded. We also excluded samples with calling rates <80 % (14 cases and 29 controls). The final analysis included 2 SNPs in the *BTNL2* gene, and 29 AIMs in 1,429 samples (486 sarcoidosis cases and 943 controls). Mean call rate in the final data set for both SNPs and samples was 99.8 %.

Statistical analysis

BTNL2—We tested each SNP for association with sarcoidosis risk using the Cochran–Armitage trend test of an additive genetic model as implemented in the PLINK software (Purcell et al. 2007). We used logistic regression analysis (PROC LOGISTIC, SAS statistical software version 9.1.3, SAS Institute Inc., Cary, NC, USA) to estimate per allele odds ratios (ORs), odds ratios for heterozygosity and homozygosity of the risk alleles, and 95 % confidence intervals. We controlled for age, geographical region of residence (Northeast, South, Midwest, West), nativity (US, foreign country), and percent African ancestry. ORs of haplotypes of SNPs in *BTNL2* were estimated using an exception substitution approach (Stram et al. 2003; Zaykin et al. 2002) which estimates the probabilities of all possible haplotype configurations of each individual in the sample, conditional on their genotype and case–control status. Haplotypes with an estimated frequency of <5 % were pooled in a single group and the most common haplotype was used as the reference haplotype.

Admixture analysis—We estimated individual percent African ancestry using a Bayesian approach as implemented in the ADMIXMAP software (Hoggart et al. 2003; McKeigue et al. 2000). Prior allele frequencies of the African and European populations were taken from the International HapMap project (International HapMap Consortium 2003). We used the affected-only test and the ancestry-association test in ADMIXMAP to assess whether previously reported AIMs (rs35397 at 5p13, rs30533 at 5q31, rs11966463 at 6p22, and rs1462906 at 8p12) are associated with sarcoidosis in the BWHS. The affected-only test assesses whether local ancestry at a particular point in the genome differs from the overall genome-wide individual ancestry among cases. The ancestry-association test assesses

whether local ancestry in a particular genomic position differs between cases and controls. All analyses were conducted using 1,000 burn-in iterations followed for 5,000 iterations in the Markov Chain Monte Carlo algorithm.

In subanalyses, we assessed the association of the *BTNL2* SNPs and AIMs with risk of sarcoidosis among cases classified as having more severe disease: ≥4 symptoms reported at diagnosis; fair or poor general health; little or no ability to carry out daily physical activities; severe or very severe fatigue; ≥5 on the pain scale.

Results

There were 486 sarcoidosis cases and 943 controls included in the present analysis. The mean age of cases and controls was 43. Twenty-seven percent lived in the Northeast, 31 % in the South, 26 % in the Midwest, and 15 % in the West. The mean percent African ancestry was 81 % in cases and 80 % in controls.

BTNL2 gene

Both *BTNL2* SNPs (rs4424066 and rs3817963) were associated with risk of sarcoidosis when considered in separate models (Table 1). The similarity of odds ratios (OR) for heterozygous and homozygous of rs3817963 suggests a dominant model of sarcoidosis risk. However, a model that included both SNPs showed that the increased risk was due to the A-allele of the rs3817963 SNP; each copy of the A-allele was associated with a 40 % increase in risk of sarcoidosis ($p = 0.02$). Results of analyses restricted to incident cases or restricted to prevalent cases were similar to overall results (data not shown). Table 2 presents ORs for the haplotype of rs4424066 and rs3817963 using the rs4424066-G/rs3817963-G haplotype as the reference; both haplotypes carrying the A-allele of rs3817963 were associated with increased risk (Table 2).

Admixture mapping replication

We observed an inverse association between sarcoidosis risk and local African ancestry at the rs30533 AIM at chromosome 5q31 (Table 3). Each additional copy of the African allele was associated with a 43 % decreased risk of sarcoidosis based on the case-only analysis ($p = 0.007$), and a 39 % decreased risk based on the case–control analysis ($p = 0.01$). None of the other three examined genomic regions showed association between local ancestry and disease risk.

Global ancestry analysis

Mean global percent African ancestry was higher in cases than controls (81.1 vs. 80.3 % respectively, $p = 0.15$). Because we had found a significant reduction in local African ancestry around rs30533 in chromosome 5q31 among cases, we adjusted our analysis of global African ancestry for number of copies of African alleles in rs30533. After adjustment, subjects in the highest quintile of African ancestry had a 54 % increase in sarcoidosis risk (p for trend = 0.03) compared to individuals in the lowest quintile of African ancestry (Table 4). Each 10 % increase of African ancestry was associated with a significant increase of 17 % in sarcoidosis risk ($p = 0.01$).

Analyses according to measures of disease severity

We next assessed the association of each SNP with “severe” disease, as determined by self-reported symptoms (Table 5). For rs3817963, the ORs were similar to the overall estimate of 1.40 among women who reported ≥4 symptoms at diagnosis, fair or poor general health, little or no ability to carry out daily physical activities, and ≥5 on the pain scale; the OR was 1.17 among women who reported severe or very severe fatigue. For rs30533, ORs were

similar to the overall estimate of 0.70 among women who reported ≥4 symptoms at diagnosis or ≥5 on the pain scale. ORs in the smaller subgroups—women with little or no ability to carry out everyday physical activities and women who reported severe or very severe fatigue—were 1.03 (0.45–2.34) and 1.26 (0.52–1.39), respectively.

We also assessed the association of each SNP among the 97 confirmed sarcoidosis cases who provided a DNA sample. The OR for the A-allele of rs3817963, 1.48 (0.95–2.30), was similar to the overall estimate using the total 486 cases. For rs30533, the OR was equal to 0.56 (0.23–1.37), also similar to the OR with the complete set of cases.

Discussion

The *BTNL2* gene is a member of the immunoglobulin superfamily and is involved in T-cell activation (Nguyen et al. 2006; Rhodes et al. 2001). The high-risk A-allele of the rs2076530 SNP results in a truncated protein that disrupts its position in the cell membrane, interfering with normal T-cell regulation (Harding et al. 1992; Nguyen et al. 2006; Rhodes et al. 2001; Valentonyte et al. 2005). Previous studies of the *BTNL2* gene in German (Valentonyte et al. 2005) and Danish (Milman et al. 2011) samples identified rs2076530 in association with sarcoidosis risk, and the rs2076530 A-allele has been associated with disease severity in case–control studies of British (Spagnolo et al. 2007) and Dutch (Spagnolo et al. 2007; Wijnen et al. 2011) populations. Further, Coudurier et al. (2009) observed an association with the A/A genotype in three generations of a family of African ancestry exhibiting severe and highly penetrant disease. The present study of African-Americans did not confirm an association of rs2076530 (represented by its perfect proxy rs4424066) with sarcoidosis risk or disease severity. Our result is in agreement with previous findings from Rybicki et al. (2005b) and Adrianto et al. (2012). Rybicki et al. (2005b) did observe a haplotypic association of rs2076530 in combination with the nearby rs9268480 SNP in both black and white subjects. We confirmed this haplotypic association and observed that increased risk of sarcoidosis was due to the A-allele of the rs3817963 (perfect proxy of rs9268480), a SNP that has been associated with ulcerative colitis (Fisher et al. 2008; Pathan et al. 2009), a type of inflammatory bowel disease (Abraham and Cho 2009). The rs9268480 SNP was reported as having a suggestive association with sarcoidosis ($p = 1.03 \times 10^{-5}$) in a recent GWAS in African-Americans (Adrianto et al. 2012).

In the United States, blacks are more commonly and severely affected by sarcoidosis compared to whites (Rybicki et al. 1998), and are more likely to have a blood relative affected with the disease (Rybicki et al. 2001a, b). These observations are compatible with the suggestion that genes of African ancestry may be a predisposing factor for the disease. AIMs are genetic markers that show large allele frequency differences among the parental populations that gave rise to the present-day admixed population. Thus, the use of AIMs allows the estimation of individual ancestry proportions. Rybicki and colleagues (2011) conducted a genome-wide ancestry mapping scan of over 1,300 AIMs in an African-American sample comprised ACCESS subjects (ACCESS Research Group 1999), sib-pairs from the Sarcoidosis Genetic Analysis (SAGA) study (Rybicki et al. 2005a), and subjects in a family-based study based in Detroit, MI (Iannuzzi et al. 2003). Among 1,357 cases and 717 controls they found several significant associations with ancestry in multiple genomic regions including rs30533 at chromosome 5q31 (Rybicki et al. 2011). We observed a significant decrease of African ancestry around rs30533, replicating the findings of Rybicki et al. (2011). We also found that higher global percent African ancestry was associated with increased risk of sarcoidosis. The 5q31 locus contains a cluster of cytokine genes and variants which have been associated with other inflammatory disorders including Crohn's disease (Onnie et al. 2006), psoriatic arthritis (Ho et al. 2005), and asthma (Donfack et al. 2005). We did not replicate the other three regions from the admixture scan of Rybicki et al.

(2011). Power was limited because of the sample size and use of only 30 ancestry markers rather than a more dense panel (Ruiz-Narvaez et al. 2011).

The association we observed between African ancestry and sarcoidosis risk highlights an apparent paradox in view of published epidemiological data suggesting a low sarcoidosis incidence in Africa (Kotler and Zwi 1967; Scadding 1967; Van Lingen 1961). Overall, little is known about the epidemiology of sarcoidosis in Africa, and the present study lacks the data necessary to examine such patterns of disease. Previous reports have suggested that the disease is rare among Africans (Kotler and Zwi 1967; Scadding 1967; Van Lingen 1961) in contrast to the excess incidence and morbidity observed among African-Americans (Iannuzzi et al. 2007; Rybicki et al. 1998) and Afro-Caribbean immigrants to London (Edmondstone and Wilson 1985; McNicol and Luce 1985) and Paris (James and Hosoda 1994). One possibility is that the antigens that induce sarcoidosis are more likely to be found in northern latitudes (Bresnitz and Strom 1983; Hosoda et al. 1997). Alternatively, it has been suggested that factors including endemic tuberculosis and scarce diagnostic and radiologic resources have likely contributed to the underreporting of sarcoidosis in Africa (Benatar 1977, 1980; Jacyk 1984; James and Hosoda 1994). More recent reports from South Africa, (Benatar 1977, 1980) and Nigeria (Awotedu et al. 1987; Jacyk 1984; Oluboyo et al. 1987) suggest that the disease occurs with greater frequency than previously reported, and that the highest incidence of disease occurs among black Africans compared to colored (mixed-race) and white Africans (Benatar 1977, 1980).

The current study is based on sample of 486 female African-American cases of sarcoidosis. Cases and controls came from the same base population of African-American women who enrolled in the BWHS in 1995 and were drawn from across the United States. Follow-up rates of the cohort have been reasonably high, and cases who provided a DNA sample were similar to those who did not with regard to numerous characteristics and, therefore, are representative of all BWHS cases. Cases and controls were matched on geographic region of residence and nativity, and we controlled in the analysis for % African ancestry, thus reducing potential confounding due to population stratification cases of sarcoidosis were identified by self-report since it is not possible in a large study such as the BWHS to examine all women for sarcoidosis. Our validation effort in a subset of cases showed a high degree of accuracy of self-reported disease (Cozier et al. 2011). It is unlikely that misdiagnosis of sarcoidosis or incorrect reporting by participants would have been related to genetic status as neither the physicians nor women were aware of their status with regard to the genetic variants analyzed. Random misclassification would have resulted in ORs being closer to the null.

The present study lacked the clinical phenotype data necessary to explore the association with disease severity, course, or organ involvement. We were, however, able to assess associations within strata of self-reported symptoms as well as with self-reported measures of general health, ability to perform everyday activities, fatigue, and pain based on a validated instrument (Cella et al. 2010). We did not observe increased risk estimates between the SNPs analyzed and self-reported symptoms.

Conclusion

In conclusion, the present results from a population of African-American women support the role of the *BTNL2* gene and the 5q31 locus in the etiology of sarcoidosis.

Acknowledgments

This work was supported by Grant K01HL088709 from the National Heart, Lung, and Blood Institute, and grants CA058420 and CA098663 from the Division of Cancer Control and Population Science, National Cancer Institute

(http://www.cancercontrol.cancer.gov). The content is solely the responsibility of the authors and does not necessarily represent the official view of the National Institutes of Health.

References

Abraham C, Cho JH. Inflammatory bowel disease. N Engl J Med. 2009; 361:2066–2078. [PubMed: 19923578]

ACCESS Research Group. Design of a case control etiologic study of sarcoidosis (ACCESS). ACCESS Research Group. J Clin Epidemiol. 1999; 52:1173–1186. [PubMed: 10580780]

Adrianto I, Lin CP, Hale JJ, Levin AM, Datta I, Parker R, Adler A, Kelly JA, Kaufman KM, Lessard CJ, Moser KL, Kimberly RP, Harley JB, Iannuzzi MC, Rybicki BA, Montgomery CG. Genome-wide association study of African and European Americans implicates multiple shared and ethnic specific loci in sarcoidosis susceptibility. PLoS ONE. 2012; 7:e43907. [PubMed: 22952805]

Awotedu AA, George AO, Oluboyo PO, Alabi GO, Onadeko BO, Ogunseyinde O, Aghadiuno PU. Sarcoidosis in Africans: 12 cases with histological confirmation from Nigeria. Trans R Soc Trop Med Hyg. 1987; 81:1027–1029. [PubMed: 3503404]

Benatar SR. Sarcoidosis in South Africa. A comparative study in Whites, Blacks and Coloureds. S Afr Med J. 1977; 52:602–606. [PubMed: 918803]

Benatar, SR. A comparative study of sarcoidosis in white, black and coloured South Africans. Alpha Omega Publishing Ltd; Cardiff: 1980.

Bresnitz EA, Strom BL. Epidemiology of sarcoidosis. Epidemiol Rev. 1983; 5:124–156. [PubMed: 6357818]

Cella D, Riley W, Stone A, Rothrock N, Reeve B, Yount S, Amtmann D, Bode R, Buysse D, Choi S, Cook K, Devellis R, DeWalt D, Fries JF, Gershon R, Hahn EA, Lai JS, Pilkonis P, Revicki D, Rose M, Weinfurt K, Hays R. The patient-reported outcomes measurement information system (PROMIS) developed and tested its first wave of adult self-reported health outcome item banks: 2005–2008. J Clin Epidemiol. 2010; 63:1179–1194. [PubMed: 20685078]

Coudurier M, Freymond N, Aissaoui S, Calender A, Pacheco Y, Devouassoux G. Homozygous variant rs2076530 of BTNL2 and familial sarcoidosis. Sarcoidosis Vasc Diffuse Lung Dis. 2009; 26:162–166. [PubMed: 20560297]

Cozier YC, Palmer JR, Rosenberg L. Comparison of methods for collection of DNA samples by mail in the Black Women's Health Study. Ann Epidemiol. 2004; 14:117–122. [PubMed: 15018884]

Cozier YC, Berman JS, Palmer JR, Boggs DA, Serlin DM, Rosenberg L. Sarcoidosis in black women in the United States: data from the Black Women's Health Study. Chest. 2011; 139:144–150. [PubMed: 20595459]

Donfack J, Schneider DH, Tan Z, Kurz T, Dubchak I, Frazer KA, Ober C. Variation in conserved non-coding sequences on chromosome 5q and susceptibility to asthma and atopy. Respir Res. 2005; 6:145. [PubMed: 16336695]

Edmondstone WM, Wilson AG. Sarcoidosis in Caucasians, Blacks and Asians in London. Br J Dis Chest. 1985; 79:27–36. [PubMed: 3986110]

Fisher SA, Tremelling M, Anderson CA, Gwilliam R, Bumpstead S, Prescott NJ, Nimmo ER, Massey D, Berzuini C, Johnson C, Barrett JC, Cummings FR, Drummond H, Lees CW, Onnie CM, Hanson CE, Blaszczyk K, Inouye M, Ewels P, Ravindrarajah R, Keniry A, Hunt S, Carter M, Watkins N, Ouwehand W, Lewis CM, Cardon L, Lobo A, Forbes A, Sanderson J, Jewell DP, Mansfield JC, Deloukas P, Mathew CG, Parkes M, Satsangi J. Genetic determinants of ulcerative colitis include the ECM1 locus and five loci implicated in Crohn's disease. Nat Genet. 2008; 40:710–712. [PubMed: 18438406]

Harding FA, McArthur JG, Gross JA, Raulet DH, Allison JP. CD28-mediated signalling co-stimulates murine T cells and prevents induction of anergy in T-cell clones. Nature. 1992; 356:607–609. [PubMed: 1313950]

Ho P, Bruce IN, Silman A, Symmons D, Newman B, Young H, Griffiths CE, John S, Worthington J, Barton A. Evidence for common genetic control in pathways of inflammation for Crohn's disease and psoriatic arthritis. Arthritis Rheum. 2005; 52:3596–3602. [PubMed: 16255050]

Hoggart CJ, Parra EJ, Shriver MD, Bonilla C, Kittles RA, Clayton DG, McKeigue PM. Control of confounding of genetic associations in stratified populations. Am J Hum Genet. 2003; 72:1492–1504. [PubMed: 12817591]

Hosoda Y, Yamaguchi M, Hiraga Y. Global epidemiology of sarcoidosis. What story do prevalence and incidence tell us? Clin Chest Med. 1997; 18:681–694. [PubMed: 9413652]

Iannuzzi MC, Maliarik MJ, Poisson LM, Rybicki BA. Sarcoidosis susceptibility and resistance HLA-DQB1 alleles in African Americans. Am J Respir Crit Care Med. 2003; 167:1225–1231. [PubMed: 12615619]

Iannuzzi MC, Iyengar SK, Gray-McGuire C, Elston RC, Baughman RP, Donohue JF, Hirst K, Judson MA, Kavuru MS, Maliarik MJ, Moller DR, Newman LS, Rabin DL, Rose CS, Rossman MD, Teirstein AS, Rybicki BA. Genome-wide search for sarcoidosis susceptibility genes in African-Americans. Genes Immun. 2005; 6:509–518. [PubMed: 15951742]

Iannuzzi MC, Rybicki BA, Teirstein AS. Sarcoidosis. N Engl J Med. 2007; 357:2153–2165. [PubMed: 18032765]

International HapMap Consortium. The International HapMap Project. Nature. 2003; 426:789–796. [PubMed: 14685227]

Jacyk WK. Sarcoidosis in the West African. A report of eight Nigerian patients with cutaneous lesions. Trop Geogr Med. 1984; 36:231–236. [PubMed: 6506201]

James, DG.; Hosoda, Y. Epidemiology. In: James, DG., editor. Sarcoidosis and other granulomatous disorders. Marcel Dekker; New York: 1994. p. 729-743.

Kotler MN, Zwi S. Sarcoidosis in South Africa—clinical manifestations. S Afr Med J. 1967; 41:615–624.

McKeigue PM, Carpenter JR, Parra EJ, Shriver MD. Estimation of admixture and detection of linkage in admixed populations by a Bayesian approach: application to African-American populations. Ann Hum Genet. 2000; 64:171–186. [PubMed: 11246470]

McNicol MW, Luce PJ. Sarcoidosis in a racially mixed community. J R Coll Physicians Lond. 1985; 19:179–183. [PubMed: 4045768]

Milman N, Svendsen CB, Nielsen FC, van Overeem Hansen T. The BTNL2 A allele variant is frequent in Danish patients with sarcoidosis. Clin Respir J. 2011; 5:105–111. [PubMed: 21410903]

Nguyen T, Liu XK, Zhang Y, Dong C. BTNL2, a butyrophilin-like molecule that functions to inhibit T cell activation. J Immunol. 2006; 176:7354–7360. [PubMed: 16751379]

Oluboyo PO, Awotedu AA, Onadeko BO, Ukoly CO. Sarcoidosis presenting as tuberculosis: a case report. East Afr Med J. 1987; 64:504–507. [PubMed: 3691366]

Onnie C, Fisher SA, King K, Mirza M, Roberts R, Forbes A, Sanderson J, Lewis CM, Mathew CG. Sequence variation, linkage disequilibrium and association with Crohn's disease on chromosome 5q31. Genes Immun. 2006; 7:359–365. [PubMed: 16724073]

Pathan S, Gowdy RE, Cooney R, Beckly JB, Hancock L, Guo C, Barrett JC, Morris A, Jewell DP. Confirmation of the novel association at the BTNL2 locus with ulcerative colitis. Tissue Antigens. 2009; 74:322–329. [PubMed: 19659809]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MA, Bender D, Maller J, Sklar P, de Bakker PI, Daly MJ, Sham PC. PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 2007; 81:559–575. [PubMed: 17701901]

Reich D, Patterson N, De Jager PL, McDonald GJ, Waliszewska A, Tandon A, Lincoln RR, DeLoa C, Fruhan SA, Cabre P, Bera O, Semana G, Kelly MA, Francis DA, Ardlie K, Khan O, Cree BA, Hauser SL, Oksenberg JR, Hafler DA. A whole-genome admixture scan finds a candidate locus for multiple sclerosis susceptibility. Nat Genet. 2005; 37:1113–1118. [PubMed: 16186815]

Rhodes DA, Stammers M, Malcherek G, Beck S, Trowsdale J. The cluster of BTN genes in the extended major histocompatibility complex. Genomics. 2001; 71:351–362. [PubMed: 11170752]

Ruiz-Narvaez EA, Rosenberg L, Wise LA, Reich D, Palmer JR. Validation of a small set of ancestral informative markers for control of population admixture in African Americans. Am J Epidemiol. 2011; 173:587–592. [PubMed: 21262910]

Rybicki BA, Maliarik MJ, Major M, Popovich J Jr, Iannuzzi MC. Epidemiology, demographics, and genetics of sarcoidosis. Semin Respir Infect. 1998; 13:166–173. [PubMed: 9764947]

Rybicki BA, Iannuzzi MC, Frederick MM, Thompson BW, Rossman MD, Bresnitz EA, Terrin ML, Moller DR, Barnard J, Baughman RP, DePalo L, Hunninghake G, Johns C, Judson MA, Knatterud GL, McLennan G, Newman LS, Rabin DL, Rose C, Teirstein AS, Weinberger SE, Yeager H, Cherniack R. Familial aggregation of sarcoidosis. A case-control etiologic study of sarcoidosis (ACCESS). Am J Respir Crit Care Med. 2001a; 164:2085–2091. [PubMed: 11739139]

Rybicki BA, Kirkey KL, Major M, Maliarik MJ, Popovich J Jr, Chase GA, Iannuzzi MC. Familial risk ratio of sarcoidosis in African-American sibs and parents. Am J Epidemiol. 2001b; 153:188–193. [PubMed: 11159165]

Rybicki BA, Maliarik MJ, Poisson LM, Sheffer R, Chen KM, Major M, Chase GA, Iannuzzi MC. The major histocompatibility complex gene region and sarcoidosis susceptibility in African Americans. Am J Respir Crit Care Med. 2003; 167:444–449. [PubMed: 12554629]

Rybicki BA, Hirst K, Iyengar SK, Barnard JG, Judson MA, Rose CS, Donohue JF, Kavuru MS, Rabin DL, Rossman MD, Baughman RP, Elston RC, Maliarik MJ, Moller DR, Newman LS, Teirstein AS, Iannuzzi MC. A sarcoidosis genetic linkage consortium: the sarcoidosis genetic analysis (SAGA) study. Sarcoidosis Vasc Diffuse Lung Dis. 2005a; 22:115–122. [PubMed: 16053026]

Rybicki BA, Walewski JL, Maliarik MJ, Kian H, Iannuzzi MC. The BTNL2 gene and sarcoidosis susceptibility in African Americans and Whites. Am J Hum Genet. 2005b; 77:491–499. [PubMed: 16080124]

Rybicki BA, Levin AM, McKeigue P, Datta I, Gray-McGuire C, Colombo M, Reich D, Burke RR, Iannuzzi MC. A genome-wide admixture scan for ancestry-linked genes predisposing to sarcoidosis in African-Americans. Genes Immun. 2011; 12:67–77. [PubMed: 21179114]

Scadding, JG. Sarcoidosis. Eyre & Spottiswoode; London: 1967.

Schurmann M, Reichel P, Muller-Myhsok B, Schlaak M, Muller-Quernheim J, Schwinger E. Results from a genome-wide search for predisposing genes in sarcoidosis. Am J Respir Crit Care Med. 2001; 164:840–846. [PubMed: 11549543]

Spagnolo P, Sato H, Grutters JC, Renzoni EA, Marshall SE, Ruven HJ, Wells AU, Tzouvelekis A, van Moorsel CH, van den Bosch JM, du Bois RM, Welsh KI. Analysis of BTNL2 genetic polymorphisms in British and Dutch patients with sarcoidosis. Tissue Antigens. 2007; 70:219–227. [PubMed: 17661910]

Stram DO, Leigh Pearce C, Bretsky P, Freedman M, Hirschhorn JN, Altshuler D, Kolonel LN, Henderson BE, Thomas DC. Modeling and E-M estimation of haplotype-specific relative risks from genotype data for a case-control study of unrelated individuals. Hum Hered. 2003; 55:179–190. [PubMed: 14566096]

Swigris JJ, Olson AL, Huie TJ, Fernandez-Perez ER, Solomon J, Sprunger D, Brown KK. Sarcoidosis-related mortality in the United States from 1988 to 2007. Am J Respir Crit Care Med. 2011; 183:1524–1530. [PubMed: 21330454]

Valentonyte R, Hampe J, Huse K, Rosenstiel P, Albrecht M, Stenzel A, Nagy M, Gaede KI, Franke A, Haesler R, Koch A, Lengauer T, Seegert D, Reiling N, Ehlers S, Schwinger E, Platzer M, Krawczak M, Muller-Quernheim J, Schurmann M, Schreiber S. Sarcoidosis is associated with a truncating splice site mutation in BTNL2. Nat Genet. 2005; 37:357–364. [PubMed: 15735647]

Van Lingen B. Sarcoidosis in South Africa. Am Rev Respir Dis. 1961; 84((5)Pt):2–162.

Wijnen PA, Voorter CE, Nelemans PJ, Verschakelen JA, Bekers O, Drent M. Butyrophilin-like 2 in pulmonary sarcoidosis: a factor for susceptibility and progression? Hum Immunol. 2011; 72:342–347. [PubMed: 21256912]

Zaykin DV, Westfall PH, Young SS, Karnoub MA, Wagner MJ, Ehm MG. Testing association of statistically inferred haplotypes with discrete and continuous traits in samples of unrelated individuals. Hum Hered. 2002; 53:79–91. [PubMed: 12037407]

Table 1

Odds ratios (ORs) and 95 % confidence intervals (CIs) of the rs4424066 and rs3817963 SNPs in the *BTNL2* gene

SNP[a]	Risk allele frequency (%)		Individual models[b]				Combined model[e]	
	Cases (*n* = 486)	Controls (*n* = 943)	Heterozygous OR (95 % CI)[f]	Homozygous OR (95 % CI)[f]	Per allele OR (95 % CI)[f]	*p* for trend	Per allele OR (95 % CI)[f]	*p* for trend
Individual models[b]								
rs4424066 (A/G)[c]	70.6	66.0	1.20 (0.81–1.77)	1.49 (1.02–2.19)	1.23 (1.04–1.45)	0.015	1.05 (0.85–1.30)	0.65
rs3817963 (A/G)[d]	86.9	82.1	4.63 (1.39–15.4)	5.95 (1.81–19.6)	1.46 (1.16–1.82)	0.001	1.40 (1.06–1.86)	0.02

[a] Risk allele/reference allele

[b] ORs were estimated for each SNP in individual models

[c] Perfect proxy of rs2076530 in Hapmap YRI

[d] Perfect proxy of rs9268480 in Hapmap YRI

[e] ORs were estimated in a single combined model that contained both SNPs

[f] Adjusted for % of African ancestry, age, and region of residence

Table 2

Odds ratios (ORs) and 95 % confidence intervals (CIs) of haplotypes of the *BTNL2* gene

rs4424066[a]	rs3817963[b]	Haplotype frequency (%)		OR (95 % CI)[c]	*p*[d]
		Cases (*n* = 486)	Controls (*n* = 943)		
G	G	13.0	17.4	1.00 (reference)	
G	A	16.5	16.4	1.40 (1.05–1.85)	0.93
A	A	70.5	65.9	1.47 (1.17–1.85)	0.01

[a] Perfect proxy (r^2 = 1.0) of rs2076530 in Hapmap YRI

[b] Perfect proxy (r^2 = 1.0) of rs9268480 in Hapmap YRI

[c] Adjusted for % of African ancestry, age, and region of residence

[d] *p* value for difference of haplotype frequencies between cases and control

Hum Genet. Author manuscript; available in PMC 2014 July 01.

Table 3

Association of previously reported ancestral informative markers with risk of sarcoidosis in the BWHS

Marker	Chromosome	Previously reported results[a]		BWHS			
				Case-only analysis		Case–control analysis	
		aRR[b]	aOR[c]	aRR[b] (95 % CI)	*p* value	aOR[c] (95 % CI)	*p* value
rs35397	5p13	1.46	1.52	0.91 (0.63–1.32)	0.63	0.96 (0.76–1.22)	0.74
rs30533	5q31	0.67	0.72	0.57 (0.38–0.86)	0.007	0.71 (0.55–0.92)	0.01
rs11966463	6p22	1.90	1.49	0.88 (0.48–1.61)	0.69	1.11 (0.76–1.64)	0.57
rs1462906	8p12	0.65	0.73	0.85 (0.53–1.37)	0.52	1.09 (0.81–1.45)	0.59

[a] Rybicki et al. (2011)

[b] Ancestry risk ratio due to African ancestry

[c] Ancestry odds ratio due to African ancestry

Table 4
Association between global African ancestry and sarcoidosis risk in the BWHS

Quintile (median % African ancestry)	OR (95 % CI)[a]	
	Multivariable OR	Multivariable OR with additional term for rs30533
1 (66.5)	1.00 (reference)	1.00 (reference)
2 (76.0)	1.07 (0.75–1.52)	1.18 (0.82–1.70)
3 (81.9)	0.95 (0.66–1.36)	1.08 (0.75–1.58)
4 (86.6)	1.12 (0.79–1.58)	1.33 (0.91–1.94)
5 (91.5)	1.26 (0.89–1.77)	1.54 (1.04–2.27)
P for trend	0.23	0.03
OR per 10 % increase African ancestry	1.08 (0.97–1.20)	1.17 (1.03–1.33)
p value	0.16	0.01

[a]Adjusted for age and region of residence

Table 5

Association of the *BTNL2* (rs3817963) single nucleotide polymorphism and ancestral informative marker (rs30533) with self-reported measures of sarcoidosis severity

	Cases (*n*)	Per allele OR (95 % CI)	
		***BTNL2* (rs3817963)[a]**	**Ancestral Informative Marker-Ancestry association test (rs30533)**
≥4 symptoms at diagnosis[b]	133	1.31 (0.91–1.88)	0.70 (0.46–1.07)
Fair or poor health[c]	80	1.36 (0.86–2.17)	0.80 (0.46–1.39)
Little or no ability to carry out everyday physical activities[c]	35	1.31 (0.66–2.58)	1.03 (0.45–2.34)
Severe or very severe fatigue[c]	44	1.17 (0.65–2.10)	1.26 (0.57–2.79)
≥5 on the pain scale[c]	107	1.45 (0.96–2.20)	0.85 (0.52–1.39)

[a] Perfect proxy ($r^2 = 1.0$) of rs9268480 in Hapmap YRI

[b] Data obtained from BWHS Sarcoidosis Supplemental Survey. (Cozier et al. 2011)

[c] Data obtained from BWHS 2011 questionnaire