ORIGINAL ARTICLE

Identification of Immune-Relevant Factors Conferring Sarcoidosis Genetic Risk

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Abstract

Rationale: Genetic variation plays a significant role in the etiology of sarcoidosis. However, only a small fraction of its heritability has been explained so far.

Objectives: To define further genetic risk loci for sarcoidosis, we used the Immunochip for a candidate gene association study of immune-associated loci.

Methods: Altogether the study population comprised over 19,000 individuals. In a two-stage design, 1,726 German sarcoidosis cases and 5,482 control subjects were genotyped for 128,705 single-nucleotide polymorphisms using the Illumina Immunochip for the screening step. The remaining 3,955 cases, 7,514 control subjects, and 684 parents of affected offspring were used for validation and replication of 44 candidate and two established risk single-nucleotide polymorphisms.

Measurements and Main Results: Four novel susceptibility loci were identified with genome-wide significance in the European case-control populations, located on chromosomes 12q24.12 (rs653178; *ATXN2/SH2B3*), 5q33.3 (rs4921492; *IL12B*), 4q24 (rs223498; *MANBA/NFKB1*), and 2q33.2 (rs6748088; *FAM117B*). We further defined three independent association signals in the HLA region with genome-wide significance, peaking in the *BTNL2* promoter region (rs5007259), at *HLA-B* (rs4143332/HLA-B*0801) and at *HLA-DPB1* (rs9277542), and found another novel independent signal near *IL23R* (rs12069782) on chromosome 1p31.3.

Conclusions: Functional predictions and protein network analyses suggest a prominent role of the drug-targetable IL23/Th17 signaling pathway in the genetic etiology of sarcoidosis. Our findings reveal a substantial genetic overlap of sarcoidosis with diverse immune-mediated inflammatory disorders, which could be of relevance for the clinical application of modern therapeutics

Keywords: Immunochip; HLA; IL23; BTNL2; association

At a Glance Commentary

Scientific Knowledge on the Subject: Sarcoidosis is a complex granulomatous disease with unknown etiology. There is a considerable genetic contribution; however, only a small fraction of the estimated heritability is explained, with variants in HLA region conferring the largest effects.

What This Study Adds to the Field: The study successfully identified four novel genetic risk loci for sarcoidosis and refined the association signal in the HLA region to three independent signals. Our results support the hypothesis that sarcoidosis partly shares a genetic background with other immune-related diseases. Thus, our findings may inspire a revised classification of clinical disease manifestations and subphenotypes. Our results further implicate a role of genetic factors in many aspects of sarcoidosis pathogenesis, especially in IL23/Th17 signaling, and may provide hypotheses on novel therapeutic targets.

Sarcoidosis is a complex inflammatory disease of mainly the lung with an unknown etiology that affects mostly young adults (20–40 yr) (1). It is characterized by heterogeneous clinical manifestations with diverse organ involvement and disease course. Previous genome-wide association studies (GWAS) and candidate gene-driven investigations identified several genetic risk loci for sarcoidosis, such as *BTNL2* (2–4); *ANXA11* (5–8); a locus on chromosome 11q13.1 (9); and, most consistently, several loci in the *HLA* region on chromosome *6p21* (10). Some risk loci are shared for sarcoidosis and other inflammatory or autoimmune conditions. For example, allelic variation of single-nucleotide polymorphism (SNP) rs11209026 (Arg381Gln) in the *IL23R* gene locus predisposes, among others, to sarcoidosis, inflammatory bowel disease, psoriasis, and ankylosing spondylitis (11–15).

Given the large overlap of the genetic risk maps of complex inflammatory and autoimmune diseases, the Immunochip array was designed for genotyping a comprehensive set of known immune-associated variants and for fine-mapping a subset of 186 selected risk loci (16, 17). This array was successfully used before to identify novel and shared risk loci for various complex immune-related diseases (18–24). We used the Immunochip to screen 1,726 German patients with sarcoidosis and 5,482 healthy control subjects for novel sarcoidosis risk loci, followed by replication in four independent European collections (4,605 cases and 12,673 control subjects) and another three populations for replication and subphenotype analysis (Table 1).

Methods

Patients and Control Subjects
The study sample comprised 5,681 sarcoidosis cases, all of which were diagnosed according to international standards (25), 12,996 control subjects, and 684 parents of affected offspring. Analyses were performed in eight independent panels (A, B-I, B-II, C-I, C-II, D, E, and F) (Table 1). A detailed description and definition of the study sample including sarcoidosis subphenotypes is provided in the online supplement.

Immunochip Genotyping and Quality Control
DNA samples of panel A were genotyped using the Immunochip, comprising a total of 196,524 SNP assays (17). Quality control excluded 67,819 SNPs and 143 individuals from analysis. Principal component analysis revealed no population stratification in the remaining samples and no population outliers were detected. Unless described differently, genotype data of panels B, C, E, and F were generated using Sequenom Mass-ARRAY iPlex (Sequenom, Inc., San Diego, CA) (26) and Taqman technology (Applied Biosystems, Foster City, CA). In every analysis step, SNPs that had more than 5% missing data (call rate $\geqslant$ 95%),

Table 1. Study Populations

Panel	Descent	Cases			Control Subjects		
		Number*	Male (%)*	Mean (SD) Age in 2014 (*yr*)	Number*	Male (%)*	Mean (SD) Age in 2014 (*yr*)
A	European (Germany)	1,726 (1,869)	39 (40)	62.9 (11.6)	5,482 (5,600)	51 (51)	57.2 (12.6)
B-I	European (Germany)	573 (585)	49 (49)	55.7 (12.6)	3,327 (3,500)	47 (47)	48.7 (14.9)
B-II	European (Germany)	266 (307)	46 (45)	61.0 (13.7)	266 (285)	50 (50)	58.3 (17.7)
C-I	European (Czech Republic)	256 (267)	45 (46)	56.2 (13.1)	305 (330)	42 (43)	45.6 (9.6)
C-II	European (Sweden)	817 (1,121)	57 (58)	54.1 (12.4)	2,040	28	53.6 (11.2)
D	African American (USA)	781	26	47.9 (15.2)	876	22	38.9 (10.2)
E	European (Germany)	342[†]	43	52.5 (7.2)[‡]	—	—	—
F	European (Serbia)	920 (920)	47	53.3 (11.4)	—	—	—

*Data are given after quality control, with data before quality control in parentheses.
[†]Number of trios (two parents with one affected offspring).
[‡]Given for the affected offspring.

a minor allele frequency less than 1%, and exact Hardy-Weinberg equilibrium *P* less than 10^{-4} were excluded. The control subjects of panel C-III were genotyped using the Illumina Immunochip custom array (The Genome Institute, Singapore). Genotyping for panel D was performed at the Oklahoma Medical Research Foundation using the Illumina Immunochip. The online supplement provides further details.

Statistical Analysis and SNP Selection

Data filtering and any statistical analysis of genotype data was performed using PLINK v.1.07, applying logistic regression model throughout all case-control analysis, including conditional analysis using an allelic model for genotype coding (27). Most promising SNPs (excluding *ANXA11* and HLA SNPs) were selected for follow-up and joint analysis on ranking top with their *P* value ($P < 10^{-4}$) in the association analysis and a positive visual inspection of regional plots. The study design is visualized in the online supplement. HLA-haplotypes were imputed using HLA*IMP:02 with default parameters (28). All statistical tests used in this study and methods applied for *in silico* analyses are described in the online supplement.

Results

After applying conservative and established quality filters to panel A, 1,726 sarcoidosis cases, 5,482 control subjects, and 128,705 SNPs were included in the analysis of this dataset (Table 1; *see* the METHODS section) and analyzed by

a logistic regression analysis. The 44 most promising SNPs were selected based on ranked *P* values as described in the METHODS section and investigated in a joint analysis of all available European case-control panels A, B-I, B-II, C-I, and C-II (total of 4,605 cases and 12,673 control subjects) (Table 1). This approach led to the identification of four novel susceptibility loci for sarcoidosis in Europeans with genome-wide significant association signals (Table 2, Figure 1). For the respective lead variants, the effect sizes did not differ significantly between the investigated populations as assessed by the Breslow-Day test.

Novel Non-HLA Risk Loci with Genome-Wide Significance

SNP marker rs653178 on chromosome 12q24.12 was associated with the smallest *P* value in the joint analysis ($P = 1.64 \times 10^{-10}$; odds ratio [OR] [95% confidence interval (CI)], 1.19 [1.14–1.27]). The potentially causative nonsynonymous variant rs3184504 in the SH2B adaptor protein 3 (*SH2B3*) locus was in high linkage disequilibrium (LD) with the lead SNP rs653178 and strongly associated in panel A ($r^2 = 0.87$; $P = 3.42 \times 10^{-6}$; OR [95% CI], 0.83 [0.77–0.90]). For rs653178, a subphenotype-differential effect was observed for Löfgren syndrome in panel B-II ($P = 6.75 \times 10^{-3}$; OR [95% CI], 1.97 [1.20–3.24]). Complete results for the subphenotype analysis are given in Table E1 in the online supplement.

Second, in the chromosome 5q33.3 region upstream of *IL12B*, SNP rs4921492 yielded a genome-wide significant result ($P = 2.14 \times 10^{-9}$; OR [95% CI], 1.20 [1.13–1.27]) for sarcoidosis. Concerning subphenotypes,

this marker associated with nominal significance with the involvement of the central nervous system in panel F ($P = 0.035$; OR [95% CI], 1.51 [1.03–2.23]) (*see* Table E2 for complete subphenotype results). Besides the lead SNP rs4921492, five markers that are in high LD with it are strongly associated with sarcoidosis (*see* Table E3). One of those, namely SNP rs12651787 ($P = 9.59 \times 10^{-6}$; OR [95% CI], 1.20 [1.10–1.29]), is in high LD with rs1422877 ($r^2 = 0.97$), which is predicted to reside in a nuclear factor-κB binding site and to confer allele-specific binding probabilities for the transcription factor (TF) RelA.

Third, SNP rs223498 located on chromosome 4q24 near *NFKB1* showed a significant association in the joint analysis ($P = 1.28 \times 10^{-9}$; OR [95% CI], 1.19 [1.12–1.26]). In panel A, a neighboring SNP (rs227375) was associated with the same effect size (OR [95% CI], 1.19 [1.10–1.29]), but did not represent an independent signal as investigated by conditional regression analysis (data not shown). The region was sparsely covered with markers on the Immunochip (17 SNPs in a region of ±250 kb around the lead SNP). According to previous GWAS data (9), the association signal may extend beyond *MANBA* and *NFKB1* (*see* Figure E1A). For rs223498, none of the investigated sarcoidosis subphenotypes was associated significantly (*see* Table E4).

Fourth, in the *FAM117B* gene region on chromosome 2q33.2, marker rs6748088 displayed a significant *P* value in the joint analysis ($P = 2.10 \times 10^{-8}$; OR [95% CI], 1.18 [1.11–1.25]). Only four markers were located in the ±500 kb-region around the lead SNP. Consulting previous GWAS data (9) did not allow

Table 2. Association Results for Newly Discovered Sarcoidosis Risk Variants

dbSNP ID	rs653178	rs4921492	rs223498	rs6748088
Chromosome	12	5	4	2
Position	112,007,756	158,832,277	103,651,962	203,264,771
Candidate genes	*ATXN2, SH2B3*	*IL12B*	*NFKB, MANBA*	*FAM117B*
A1	G	A	C	C
A2	A	C	A	T
Panel A				
AF_{cases}	0.55	0.39	0.51	0.34
$AF_{controls}$	0.5	0.34	0.47	0.31
P value	1.36×10^{-6}	7.56×10^{-7}	6.68×10^{-6}	4.30×10^{-4}
OR (95% CI)	1.21 (1.12–1.30)	1.22 (1.13–1.32)	1.19 (1.10–1.29)	1.16 (1.07–1.26)
Panel B-I				
AF_{cases}	0.54	0.39	0.52	0.35
$AF_{controls}$	0.49	0.35	0.48	0.30
P value	8.06×10^{-3}	0.019	0.012	1.34×10^{-4}
OR (95% CI)	1.19 (1.05–1.34)	1.17 (1.03–1.33)	1.18 (1.04–1.33)	1.29 (1.13–1.48)
Panel B-II				
AF_{cases}	0.54	0.4	0.5	0.37
$AF_{controls}$	0.51	0.33	0.48	0.31
P value	ns	0.039	ns	0.048
OR (95% CI)	1.13 (0.89–1.44)	1.33 (1.04–1.71)	1.08 (0.85–1.38)	1.29 (1.00–1.67)
Panel C-I				
AF_{cases}	0.58	0.38	0.44	0.36
$AF_{controls}$	0.48	0.36	0.49	0.32
P value	8.94×10^{-4}	ns	ns	ns
OR (95% CI)	1.49 (1.18–1.89)	1.11 (0.87–1.41)	0.82 (0.64–1.03)	1.20 (0.94–1.54)
Panel C-II				
AF_{cases}	0.50	0.38	0.54	0.32
$AF_{controls}$	0.48	0.35	0.48	0.30
P value	ns	ns	1.08×10^{-5}	ns
OR (95% CI)	1.12 (0.99–1.26)	1.14 (0.94–1.39)	1.30 (1.16–1.46)	1.10 (0.95–1.27)
Joint analysis				
P CMH	1.64×10^{-10}	2.14×10^{-9}	1.28×10^{-9}	2.10×10^{-8}
OR (95% CI)	1.19 (1.14–1.27)	1.20 (1.13–1.27)	1.19 (1.12–1.26)	1.18 (1.11–1.25)

Definition of abbreviations: AF = allele frequency; CI = confidence interval; CMH = Cochran-Mantel-Haenzel test; dbSNP = National Institutes of Health SNP database; ns = not significant; OR = odds ratio. The position of the respective lead variant is given according to human genome build 19. OR refers to allele 1 (A1), and *P* values are presented for the logistic regression model using an allelic model for genotype coding. The threshold for genome-wide significance ($P < 5 \times 10^{-8}$) in the CMH test (*P* CMH) was applied to define a true association.

the restriction of the association signal to a specific gene encoded in the region, but suggests a second independent signal in the genomic region (*see* Figure E1B). Conditional regression analysis in this dataset suggests an independent signal, represented by rs17469010 ($P = 0.002$; OR [95% CI], 1.68 [1.21–2.33]). Subphenotype analysis did not reveal any significant differential effects for this marker (*see* Table E5).

The complete results for the 44 selected SNPs, including *P* values for the Breslow-Day test, are listed in Tables E6 and E7. None of the four novel risk variants showed a significant association in the African American sample (panel D). Power calculations revealed limited power to replicate the findings in panel D (rs653178, 14.6%; rs4921492, 42.7%;

rs223498, 26.1%; rs6748088, 21.4%). In the German trio panel E, one of the newly identified risk SNPs (rs4921492) showed nominal significance ($P = 0.039$; OR [95% CI], 1.27 [1.01–1.59]) (*see* Table E8). The statistical power to detect the three remaining association signals in panel E was low with 17.6% (rs653178) and 14.0% (rs223498 and rs6748088), respectively.

Candidates and Secondary Signals
One SNP in the *TYK2* gene region, rs34536443, was associated with nearly genome-wide significance in the joint analysis of the European case-control panels ($P = 5.48 \times 10^{-8}$; OR [95% CI], 0.64 [0.55–0.76]) (*see* Table E3) and showed the same trend in the German trio panel E ($P = 0.086$; OR, 0.55 [0.27–1.10]) (*see* Table E4). It causes an amino acid change in the

TYK2 protein (Pro1104Ala) and has therefore a high potential to be functionally relevant. No data were available for this SNP for the African American sample (panel D). Subphenotype analysis in panel F suggests an association with fibrosis compared with patients with no fibrosis ($P = 0.01$; OR [95% CI], 4.59 [1.33–15.76]; allele frequency$_{fibrotic}$ = 0.06; allele frequency$_{nonfibrotic}$ = 0.01).

Furthermore, two SNPs in the known sarcoidosis risk locus *IL23R* on chromosome 1p31.3 were associated with genome-wide significance in panel A (rs12069782: $P = 4.20 \times 10^{-8}$; OR [95% CI], 1.30 [1.18–1.43] and rs12090164: $P = 4.51 \times 10^{-8}$; OR [95% CI], 1.30 [1.18–1.42]) (*see* Tables E6 and E7), of which rs12090164 was omitted from follow-up because of complete LD ($r^2 = 1$). Subphenotype analysis did not reveal any subphenotype-differential effects (*see* Table E9). Conditional regression analysis showed that the association signal of rs12069782 was independent of the association previously reported (11, 29). The online supplement provides more details. The lead SNP rs12069782 was subjected to replication in the European case-control populations (panels B-I, B-II, C-I, and C-II). In this analysis the ORs ranged from 1.03 to 1.46, with $P = 3.07 \times 10^{-10}$ (OR [95% CI], 1.24 [1.16–1.33]) in the joint analysis of panels A, B, and C (*see* Tables E6 and E7). However, the association was not confirmed in the African American panel D (power, 68.3%) or the German trio panel E (power, 14.7%) (*see* Table E8).

Risk Variants in the HLA Region
The combined analysis of single markers in the HLA region and imputed classical HLA alleles in panel A revealed a total of 1,172 associated markers of the extended HLA region (chr6: 25–35 Mb; $P < 5.0 \times 10^{-8}$), comprising three independent association signals (*see* online supplement for complete results).

The BTNL2 Region
The strongest association in the HLA region was represented by marker rs5007259 in the *BTNL2* promoter region ($P = 1.55 \times 10^{-35}$; OR [95% CI], 0.60 [0.55–0.65]) (Figure 2). In a recently published association study comprising panel D (samples of African American [AA] origin) and additional sarcoidosis patients and control subjects of
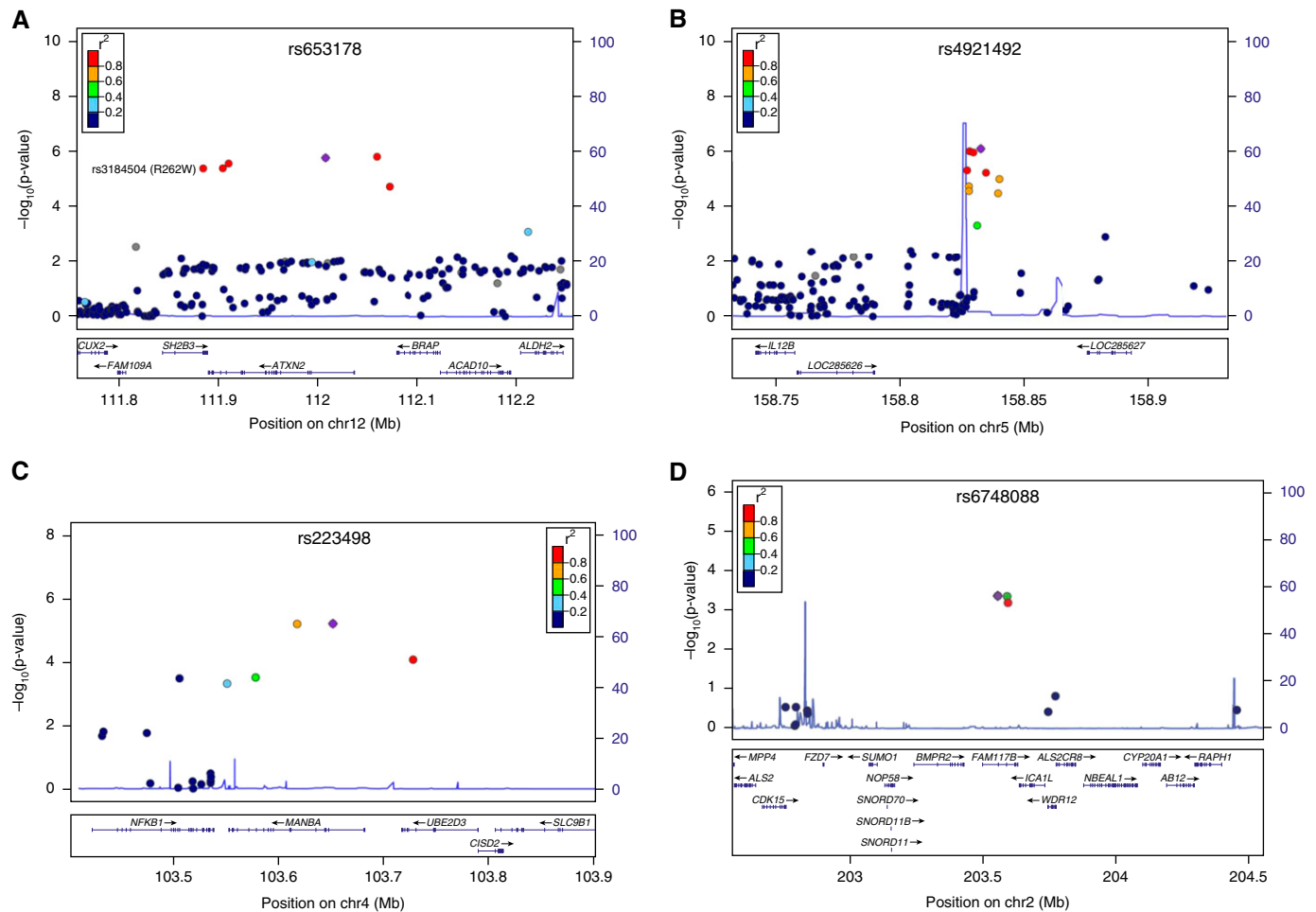
Figure 1. Association signals of newly discovered sarcoidosis risk regions panel A that showed genome-wide significance in the joint analysis of the European case-control panels A, B-I, B-II, C-I, and C-II on (A) chromosome 12q24.12 (lead single-nucleotide polymorphism [SNP] rs653178, nonsynonymous SNP rs3184504), (B) 5q33.3 (lead SNP rs4921492), (C) 4q24 (lead SNP rs223498), and (D) 2q33.2 (lead SNP rs6748088). The respective lead SNPs are marked by *blue diamonds*. The strength of linkage disequilibrium (r^2) between the lead SNP and surrounding markers and the recombination rate is given according to 1,000 Genomes data (phase I, release March 2012) and is indicated by *red* to *yellow* coloring and the *light blue line*, respectively. Positions shown are according to National Center for Biotechnology Information's build 37(hg19).

European American (EA) origin (30), marker rs5007259 was associated with nominal significance in both subsets ($p_{AA} = 3.80 \times 10^{-3}$; $p_{EA} = 7.05 \times 10^{-7}$). Table E10 provides complete results on overlapping signals in the HLA region. Subphenotype analysis of rs5007259 revealed a nominally significant association with Löfgren syndrome, with OR ranging from 0.37 to 0.52. Complete results for the subphenotype analysis of this marker are given in Table E11.

The *BTNL2* region was densely covered by the Immunochip, allowing fine-mapping of the major signal to the putative promoter region of *BTNL2* (see Figure E2). The previously reported functional splice site variant rs2076530 was significantly associated (panel A; $P = 9.64 \times 10^{-25}$; OR [95% CI],

0.65 [0.60–0.70]), however no longer significant after including the allelic dosage for rs5007259 as a covariate (i.e., conditioning on rs5007259) ($P_{cond} = 0.097$). In contrast, the association of rs5007259 remained highly significant ($P_{cond} = 5.00 \times 10^{-13}$). According to FuncPred, the most strongly associated variants in the promoter region are predicted to reside in TF binding sites.

Analysis of HLA-haplotypes using logistic regression analysis revealed a significant association of HLA-DRB1-haplotypes (*0101: $P = 1.42 \times 10^{-18}$; OR [95% CI], 0.48 [0.41–0.57] and *0301: $P = 4.99 \times 10^{-19}$; OR [95% CI], 1.65 [1.48–1.84]) with sarcoidosis, a finding that has most consistently been reported (reviewed in Reference 31). HLA-DRB*0301 was associated also in the small

sample of patients with Löfgren syndrome ($P = 2.25 \times 10^{-13}$; OR [95% CI], 4.20 [2.86–6.86]), confirming previous findings (32). The *BTNL2* SNPs rs5007259 remained significantly associated after conditioning on HLA-DRB1*0101 ($P = 1.63 \times 10^{-23}$; OR [95% CI], 0.65 [0.60–0.71]) and HLA-DRB1*0301 ($P = 5.63 \times 10^{-25}$; OR [95% CI], 0.64 [0.59–0.70]) in our study population. Vice versa, the association of both haplotypes was diminished by conditioning on rs5007259 ($P = 2.29 \times 10^{-8}$; OR [95% CI], 0.61 [0.52–0.73] and $P = 5.43 \times 10^{-7}$; OR [95% CI], 1.34 [1.20–1.51]).

HLA-B and HLA-DPB1

When conditioning all markers and the imputed haplotypes in the HLA region on
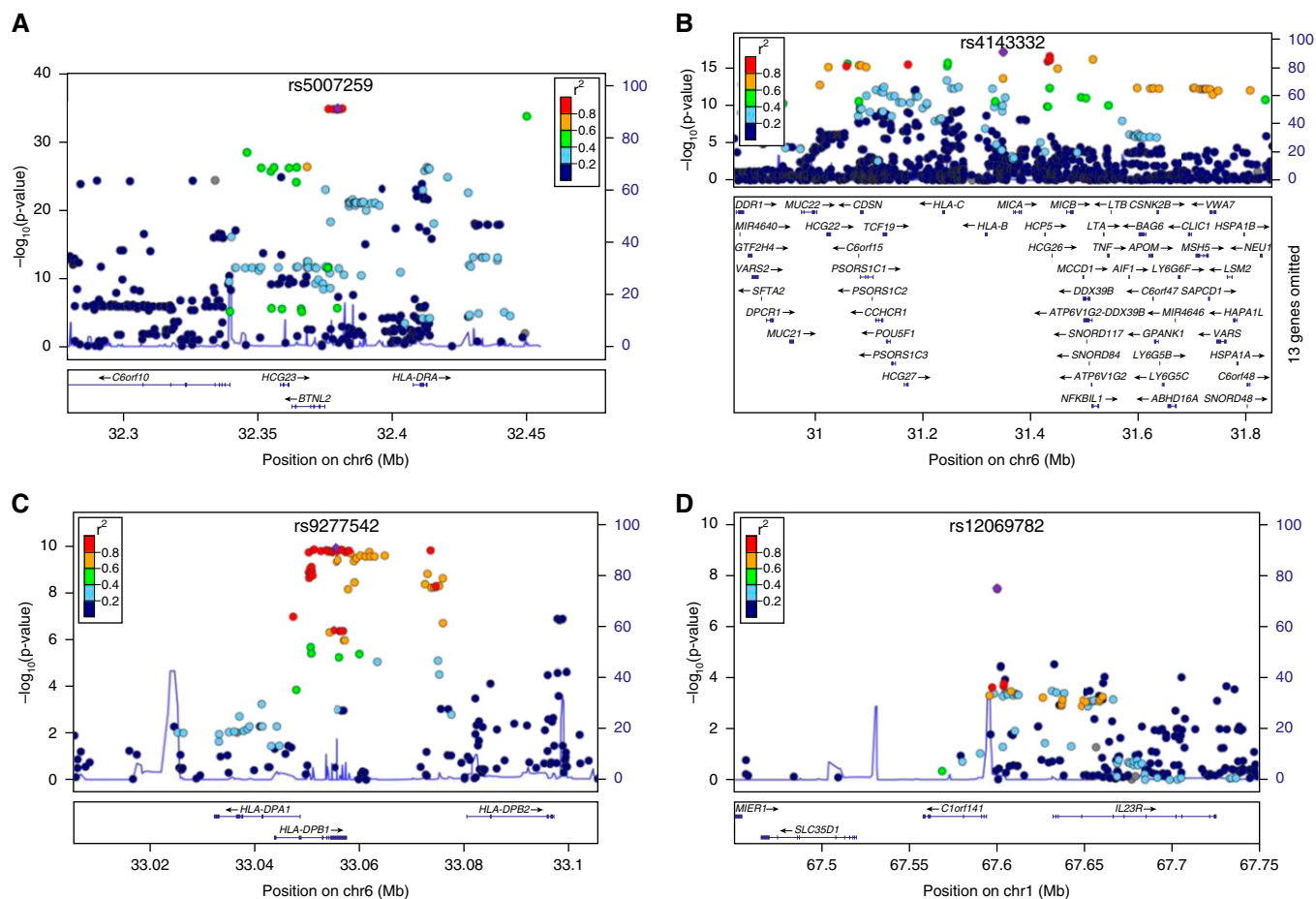
Figure 2. Association signals with genome-wide significance in the screening panel A. (*A*) BTNL2 (rs5007259), (*B*) HLA-B (rs4143332), (*C*) HLA-DPB1 (rs9277542), and (*D*) IL23R (rs12069782). For *B* and *C*, the depicted *P* values are derived from the sequential conditional regression analysis, conditioned on single-nucleotide polymorphism rs5007259 (*B*) and on rs5007259 and rs4143332 (*C*). See Figure 1 for descriptions.

rs5007259, marker rs4143332, which is located upstream of *HLA-B*, yielded the smallest *P* value ($P_{cond} = 7.73 \times 10^{-18}$; OR [95% CI], 1.64 [1.47–1.84]) (Figure 2). The most significantly associated haplotype HLA-B*0801 showed an association signal of similar strength ($P_{cond} = 2.56 \times 10^{-17}$; OR [95% CI], 1.63 [1.45–1.82]). Subphenotype analysis of rs4143332 revealed a significant difference in allele frequency between patients affected by acute and chronic sarcoidosis (OR, ≤2.21), and an even stronger differential effect for Löfgren syndrome (OR, ≤2.85). Table E12 provides complete results of the subphenotype analysis for this marker.

Conditioning on both rs5007259 and rs4143332 revealed a third independent association signal with genome-wide significance in the HLA region that is

represented by SNP rs9277542 ($P_{cond} = 1.13 \times 10^{-10}$; OR [95% CI], 1.32 [1.21–1.44]) (Figure 2). In panel F, this marker displayed a differential effect for skin involvement ($P = 7.13 \times 10^{-3}$; OR [95% CI], 0.62 [0.44–0.88]). The complete results for the subphenotype association analysis are given in Table E13. Marker rs9277542 is located in the 3′-UTR of *HLA-DPB1*. None of the surrounding highly associated markers confer an amino acid change in the encoded protein, but functional predictions suggest a role in expression regulation via allele-specific miRNA binding (*see* Table E14) and alternative splicing according to SNPinfo (33). The online supplement and Data File E1 provide results of imputed HLA-haplotype analysis, subphenotype analysis, and complete information on the association of HLA haplotypes.

Association signals of established sarcoidosis risk variants are given in the online supplement.

Protein Network Analysis
A network analysis of potentially affected gene products was performed using STRING (34). Proteins that were included in the analysis were selected based on the association and *in silico* analysis presented in this manuscript (i.e., BTNL2, HLA-DPB1, HLA-B, MICA, ANXA11, IL23R, SH2B3, CRIP1, IL12B, MANBA, NFKB1, and FAM117B) or were previously described or hypothesized as potentially affected (i.e., TNF, HLA-DRB1, HLA-DQB1, OS9, CYP27B1, KCNK4, CCDC88B, and RAB23). The analysis identified one prominent protein network, comprising key molecules of the IL12/IL23 signaling pathways (Figure 3).

Sharing of Risk Loci and Cumulative Heritability

Our study demonstrated a considerable sharing pattern of sarcoidosis susceptibility factors, especially of the IL23/IL12 signaling pathway with other disorders and phenotypes, such as ankylosing spondylitis, inflammatory bowel disease, and psoriasis (*IL23R*, *IL12B*, and *TYK2*); rheumatoid arthritis (*IL23R* and *TYK2*); and multiple sclerosis (*IL12B* and *TYK2*; reviewed in Reference 17). However, the exact association patterns are not identical for these conditions as illustrated in a comparison of the *IL12B* and *IL23R* signal for sarcoidosis, psoriasis, and Crohn disease (35) (*see* Figure E3). Sequential conditional logistic regression analysis, calculation of LD, and test for epistasis proved the independence of the 11 sarcoidosis risk variants located in the *BTNL2*, *HLA-B*, *HLA-DPB1*, *ANXA11*, *IL23R*, *ATXN2*, *IL12B*, *MANBA*, *FAM117B*, chromosome 11q13.1, and *RAB23* regions (*see* Figure E4). However, assuming a prevalence of 40 per 100,000, the cumulative heritability of the respective 11 lead variants was estimated to be only 2.78% (*see* Table E15).

Discussion

In our study, we successfully screened the largest sarcoidosis case-control study population to date for novel risk factors and identified chromosomes 12q24.12
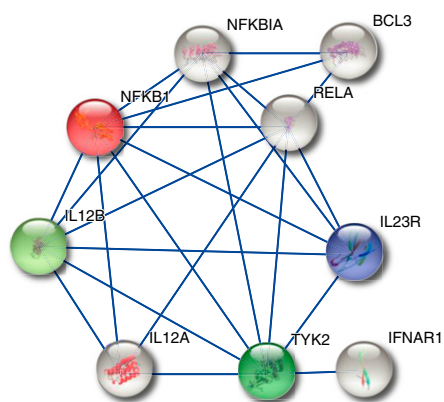
Figure 3. Protein interaction network, which was generated using STRING based on (*1*) coexpression of the respective transcripts, (*2*) experiments, and (*3*) information from (in parts) curated pathway databases, applying a confidence score >0.7. *Colored nodes* represent proteins that are or may be affected by sarcoidosis risk variants.

(*ATXN2/SH2B3*), 5q33.3 (near *IL12B*), 4q24 (*MANBA/NFKB1*), and 2q33.2 (*FAM117B*) as susceptibility regions for sarcoidosis in the investigated European samples. We further found a novel, independent association signal on chromosome 1p31.3 (*IL23R*). Because of the extraordinary sample size and the applied level of significance, our findings are highly reliable to represent true genetic risk factors for sarcoidosis in Europeans. However, the analysis of the associated markers in an African American sarcoidosis population was not successful, which could have power (<68% throughout all tests), genetic, and functional reasons. This discrepancy points out the need for explicit transancestry association studies to get insight into the genetic architecture of sarcoidosis across populations of diverse ancestry. Analysis of sarcoidosis subphenotypes revealed several findings with nominal significance. However, because of the large numbers of tests, most of these results would not remain significant after correction for multiple testing and thus have to be investigated in independent samples for validation. Therefore these findings are not discussed in detail here.

The identified sarcoidosis susceptibility loci harbor genetic variants that may be functionally relevant in the context of sarcoidosis pathogenesis, affecting either known or novel players. First, variant rs3184504 on chromosome 12q24.12 induces an amino acid change (R262W) in the SH2B3 protein, an intriguing candidate. SH2B3 is involved in B-cell proliferation (36) and the endothelial response to TNF (37) and has not yet been implicated in sarcoidosis pathogenesis. The rs3184504*A risk allele is associated with stronger activation of the NOD2 recognition pathway in response to lipopolysaccharide and muramyl dipeptide, suggesting SH2B3 may play a role in protection against bacterial infection (38). Second, among the associated markers in the chromosome 5q33.3 region, SNP rs1422877 confers allele-specific binding probabilities for the TF RelA, which could influence the expression of neighboring genes (e.g., of the *IL12B* gene that encodes the p40 subunit of the IL12 and the IL23 receptors). Third, the lead SNP in the chromosome 1p31.3 region is located in the putative promoter region of the *IL23R* gene and might therefore influence *IL23R* expression.

Fourth, the associated variants on chromosome 2q33.2 could affect the expression of many genes, among them BMPR2, which is involved in the regulation of inflammatory processes in the lung (39) and has been suggested as a core mediator in sarcoidosis pathogenesis in comparison with idiopathic pulmonary fibrosis (40). Fifth, allelic variation of the candidate risk SNP rs34536443 (*TYK2* Pro1104Ala) is known to influence Th2 lymphocyte polarization (41). The risk allele "C" reduces TYK2 activity in T lymphocytes and shifts the cytokine secretion toward a Th2 cytokine profile. This shift is also observed in severe sarcoidosis with progressing pulmonary fibrosis (42). Strikingly, the rare rs34536443-C allele is strongly associated with fibrosis among patients with sarcoidosis in panel F. Because of the large effect size (OR, 4.6), this finding provides potential for clinical application as a marker for high-risk patients, given a successful independent replication. Functional hypotheses on risk variants in the remaining novel susceptibility regions are given in the online supplement. Because the identification of genetic defects and deranged pathways does not necessarily imply a direct link to immunopathogenesis or therapy, further experimental work is necessary to assess these previously mentioned hypotheses and to clarify the potential role of the associated variants in the specific molecular processes in sarcoidosis pathogenesis and whether they represent useful targets in a therapeutic approach.

A network analysis of the potentially affected gene products defined in this study identified one prominent protein network, comprising key molecules of the IL12/IL23 signaling pathway (reviewed in Reference 17). From these data, we hypothesize that the IL12/Th1 and IL23/Th17 signaling pathways could be affected by the described risk alleles. Both Th1 and Th17 cells are present in granulomas of patients with sarcoidosis and are known to play a crucial role in disease process (43, 44). This is in line with emerging reports on the role of Th17 T cells in sarcoidosis and confirms Th1 cells as well-established players in disease pathogenesis (44, 45).

Of note, the two loci *IL12B* and *IL23R* are shared risk loci for sarcoidosis,

the skin disease psoriasis, and the granulomatous Crohn disease, yet with differing association patterns. Given a more detailed genetic investigation, this information could be of value, because targeting the IL12/IL23 signaling pathway using the p40 antibody ustekinumab is approved in psoriasis treatment and also seems promising in the therapy of severe Crohn disease (46, 47). However, for sarcoidosis no such positive effect was observed (48). Because genetic data were not incorporated in this ustekinumab trial in sarcoidosis, we hypothesize that genetic information might aid the identification of a subset of patients with sarcoidosis that may have a higher chance to respond to the treatment, alike reported for a variant in the *TNF* locus and anti-TNF treatment (49). Functional and clinical studies are necessary to evaluate the usefulness of ustekinumab in such a genetically defined subcohort.

The HLA region has long been known and is most consistently reported to be associated with sarcoidosis. Our group had reported the association of *BTNL2* and suggested the splice variant rs2076530 as the underlying risk factor before (2). Since then, this association has been replicated in other European and non-European populations (4, 50–55). However, in this study the most strongly associated variants were located in the *BTNL2* promoter region and are predicted to reside in TF binding sites. They may therefore influence expression of *BTNL2* by altering TF binding. Thus, this result may point to a different underlying mechanism, although it confirms BTNL2 as a risk factor for sarcoidosis.

The second independent signal in the HLA region peaked near HLA-B with the common haplotype HLA-B*0801 showing the strongest effect, with lead SNP rs4143332 being significantly associated with acute sarcoidosis and Löfgren syndrome, which is in line with previous findings (56, 57). It is part of the so-called "8.1 ancestral haplotype" (HLA A*0101: Cw*0701: B*0801: DRB1*0301: DQA1*0501: DQB1*0201), which is common in Europeans and includes a large number of genes related to the immune system. The 8.1 ancestral haplotype has been repeatedly reported in the context of sarcoidosis susceptibility and progression (56, 57) and is associated with several other immune-related diseases (58), such as systemic lupus erythematosus and primary sclerosing cholangitis (21, 59).

The third signal in the HLA-region is located in the 5′-UTR region of *HLA-DPB1*. This gene encodes a subunit of the HLA-DP receptor that plays a central role in the immune system by presenting peptides derived from extracellular proteins. *HLA-DPB1* genotypes had been linked to sarcoidosis before (60–63) and the Glu69 variant (rs1042140) is known to be associated with chronic beryllium disease, a phenocopy of sarcoidosis (64). In our study population, none of the Glu69-containing haplotypes were associated with genome-wide significance (data not shown). Instead, this is the first report that locates this association at *HLA-DPB1* to the 5′-UTR region of the gene, where the associated markers are predicted to affect miRNA binding and alternative splicing. The online supplement provides information for further functional hypotheses on associated variants. Overall, the genetic findings reported here are highly reliable, setting a most solid ground for subsequent experimental validation.

Altogether based on our study and previous findings, we defined 11 sarcoidosis risk loci (*BTNL2, HLA-B, HLA-DPB1, ANXA11, IL23R, SH2B3/ATXN2, IL12B, NFKB1/MANBA, FAM177B*, chromosome 11q13.1, and *RAB23*). However, the cumulative heritability of the 11 lead variants was estimated to be only 2.78%. This means that additional genetic risk factors for sarcoidosis remain to be discovered, which may include common variants with even smaller effects or risk alleles with lower frequencies, which were not targeted with the SNP selection strategy applied in this study. The detected loci show a considerable sharing pattern with other disorders and phenotypes. This overlap is supportive of the hypothesis that sarcoidosis shares a common genetic background with other immune-related diseases. Thus, our findings may inspire a revised classification of clinical disease manifestations and subphenotypes and may provide hypotheses for novel therapeutic targets. ∎

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