

High-Density Genetic Mapping Identifies New Susceptibility Variants in Sarcoidosis Phenotypes and Shows Genomic-driven Phenotypic Differences

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Abstract

Rationale: Sarcoidosis is a multisystem disease of unknown cause. Löfgren's syndrome (LS) is a characteristic subgroup of sarcoidosis that is associated with a good prognosis in sarcoidosis. However, little is known about its genetic architecture or its broader phenotype, non-LS sarcoidosis.

Objectives: To address the genetic architecture of sarcoidosis phenotypes, LS and non-LS.

Methods: An association study in a white Swedish cohort of 384 LS, 664 non-LS, and 2,086 control subjects, totaling 3,134 subjects using a fine-mapping genotyping platform was conducted. Replication was performed in four independent cohorts, three of white European descent (Germany, n = 4,975; the Netherlands, n = 613; and Czech Republic, n = 521), and one of black African descent (United States, n = 1,657), totaling 7,766 subjects.

Measurements and Main Results: A total of 727 LS-associated variants expanding throughout the extended major histocompatibility complex (MHC) region and 68 non-LS-associated variants located in the MHC class II region were identified and confirmed. A shared overlap between LS and non-LS defined by 17 variants located in the MHC class II region was found. Outside the MHC region, two LS-associated loci, in *ADCY3* and between *CSMD1* and *MCPH1*, were observed and replicated.

Conclusions: Comprehensive and integrative analyses of genetics, transcription, and pathway modeling on LS and non-LS indicates that these sarcoidosis phenotypes have different genetic susceptibility, genomic distributions, and cellular activities, suggesting distinct molecular mechanisms in pathways related to immune response with a common region.

Keywords: genetic epidemiology of sarcoidosis; Löfgren's syndrome; non-Löfgren's syndrome; genome-wide associations; Immunochip

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At a Glance Commentary

Scientific Knowledge on the

Subject: The genetics in subgroups of patients with sarcoidosis have not been previously investigated.

What This Study Adds to the

Field: This study delineates the genetic architecture of the specific subgroup with Löfgren's syndrome.

Sarcoidosis is a systematic inflammatory disease of unknown cause that is characterized by noncaseating granulomas. The main affected organ is the lung, which is engaged in more than 90% of patients and about a quarter develop pulmonary fibrosis with permanent dysfunction (1). Löfgren's syndrome (LS) is an acute form of sarcoidosis, characterized by bilateral lymphadenopathy on chest radiography (in some cases with parenchymal infiltrates), erythema nodosum, and/or bilateral ankle arthritis or distinct periarticular inflammation (2). Patients with LS have a better prognosis compared with patients without LS, as evaluated 2 years after disease onset. In particular, patients with LS carrying the *HLA-DRB1*03* allele run a good disease course (3).

Sarcoidosis has a peak incidence in the third and fourth decade of life, with a higher disease rate in females, and has major effects on quality of life, the ability to work, and basic activities of daily living (4, 5). In Sweden, the incidence rate of sarcoidosis has been estimated at approximately 64 per 100,000 persons (6) and is highly represented by LS (7) with an estimate of 15 per 100,000 persons yearly as observed in our respiratory unit.

In the present study, we aimed to identify genetic variants linked specifically with two sarcoidosis phenotypes, LS and non-LS, in a sample of white Swedish

persons. Replication analysis of identified genetic variants was conducted in independent cohorts with similar phenotypic characterization of European ancestry from Germany, the Netherlands, and Czech Republic and of African American ancestry from the United States. A genome-wide association approach was adopted using a high-density genetic mapping technique that has been used across a range of autoimmune and inflammatory diseases (8–11) but so far not for sarcoidosis phenotypes.

Methods

Study Design and Participants

All studies had protocols approved by local institutional review boards. All participants in Sweden, Germany, the Netherlands, Czech Republic, and United States provided written informed consent and gave permission to use their DNA for research purposes. Baseline measures of clinical and demographic characteristics were measured at the time of the participants' enrollment in each study. Details of study cohorts, genotyping, and quality control of genetic data are available in the METHODS section of the online supplement.

Statistical Analysis

Association study of LS and non-LS. For both LS and non-LS, logistic regression analysis of genotyped and imputed single-nucleotide polymorphisms (SNPs) was performed with expected genotype count using the posterior probabilities. Analyses were adjusted for sex and were performed using PLINK v1.07 (12) on ImmunoChip data. Genome-wide significance was defined as genomic control-corrected P_{GC} less than 5×10^{-8} and a suggestive significance threshold of P_{GC} greater than 5×10^{-8} and less than 5×10^{-5} was

established for identifying susceptible variants. The suggestive P value was chosen based on published works (9, 11, 13) using ImmunoChip data and proposed threshold significance P_{Ichip} less than 10^{-4} . Moreover, conditional analysis on the lead SNP was performed as to evaluate for other potential variants. Additionally, conditional analysis on *HLA-DRB1* alleles was also performed. Each *HLA-DRB1* allele was analyzed as a covariate along with sex and was coded as positive (assigned a "1") or negative (assigned a "0") for the allele in question.

Replication of significant SNPs.

Replication of associated SNPs was conducted in four independent cohorts, three of white European descent and one of black African descent, totaling 7,766 subjects. Specifically, in samples from Germany (64 LS, 413 non-LS, and 4,498 control subjects) and from African Americans (781 non-LS and 876 control subjects), SNPs identified at P_{GC} less than 5×10^{-5} in the discovery cohort were extracted from an available dataset typed on the ImmunoChip. In subjects from the Netherlands (241 LS, 180 non-LS, and 192 control subjects) and Czech Republic (47 LS, 263 non-LS, and 211 control subjects), the top SNP associated with LS and non-LS, respectively, was genotyped by TaqMan assay. A nominal P value threshold for replication significance was set to P less than 0.05 with Bonferroni correction.

Meta-analysis of associated SNPs. We conducted meta-analysis by combining association results from all cohorts.

An inverse variance weighting approach on regression estimates and standard errors was used as implemented in the Metafor (14) R-package for high and suggestive signals associated with LS and non-LS, respectively. We also applied genomic control to the discovery cohort (given that the inflation factor, lambda, was >1.00 [i.e., 1.16 in LS, and 1.17 in non-LS]) by

The Swedish cohort was funded by Swedish Heart-Lung Foundation, by the Swedish Medical Research Council, and through the regional agreement on medical training and clinical research between Stockholm County Council and the Karolinska Institutet. The Czech Republic cohort was funded by grant IGA_LF_2015_020 (M.P.). The German study was supported by the Federal Ministry for Education and Research in Germany through the National Genome Research Network, within the framework of the e:Med (sysINFLAME) research and funding concept (01ZX1306A), the Genome Research Network "MooDS," and the PopGen 2.0 network (01EY1103); by the German Research Foundation through grants FI 1935/1-1, MU 692/8-1, and BR 1912/6-1; through the Clusters of Excellence "Inflammation at Interfaces" and "ImmunoSensation"; by the Heinz Nixdorf Foundation; and by the Else-Kröner-Fresenius-Stiftung (Else Kröner-Exzellenzstipendium 2010_EKES.32). The African American study was supported by National Institutes of Health grant P30GM110766.

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multiplying standard errors by the square root of lambda. For both phenotypes, we calculated a meta-analysis log odds ratio (OR), 95% confidence interval (CI), meta-*P* value, along with model statistic measures, amount of heterogeneity in the true log effect size (τ^2), heterogeneity *P* value ($\tau^2 P$), and total variability in effect size (I^2). Fixed-effect and random-effect models were used for carrying out meta-analysis. We established a significance threshold of meta-*P* less than 5×10^{-8} as genome-wide significant.

Gene-centric analysis. To explore the genetic effects of variants located within a gene including those in introns, coding, 5' untranslated region (5' UTR), and 3' UTR, we performed a genome-wide association gene-based approach for LS and non-LS, respectively, using the versatile gene-based association test as implemented in VEGAS (Versatile Gene-based Association Study) (15) software, which requires genic SNPs and individual marker *P* values from genome-wide association test to compute gene-based *P* value. From the association summary statistics of LS and non-LS, we extracted 48,740 SNPs tagging 6,840 genes across the genome and used the genomic-controlled *P* value (P_{GC}) as *P* value entry. To account for LD structure, the HapMap phase 2 CEU population (Utah residents with ancestry from northern and western Europe) was used as reference along with the top SNP option. A Bonferroni-corrected threshold of *P* less than 7.3×10^{-6} (0.05/6840) was used for genome-wide significance and *P* less than 0.05 for nominal significance.

Integrative Analyses on Significant Findings

Bioinformatic analysis for refinement of associated variants. To explore the potential functionality of genetic variants associated with LS and non-LS and to substantiate their associations with the phenotypes in question, we evaluated genetic variant involvement in expression quantitative trait loci (eQTL), which are known to be directly linked to DNA variation. Using public/published eQTL databases (16–25), we classified a SNP as *cis*-eQTL SNP if it was found associated with eQTL at *P* less than 0.05. For SNPs with no direct *cis*-eQTL association, we computed the linkage disequilibrium (LD; defined by $R^2 > 0.8$ and $D' = 1$) using HapMap and 1,000

Genomes datasets to establish whether the SNP was near a *cis*-eQTL SNP. Additionally, to investigate whether a SNP had a role in machineries of gene regulation, we used available datasets from ENCODE (the Encyclopedia of DNA Elements) and the National Institutes of Health Roadmap Epigenomics Mapping Consortium. Using RegulomeDB (26) and HaploReg v2 (27) databases, we obtained measured/predicted data for all SNPs in question across various tissues and cell types. We classified a SNP as a regulatory SNP if it had at least one measured/predicted regulatory function present across collected data.

Enrichment and pathway analyses of associated variants. Enrichment and pathway analyses were conducted based on significant confirmed variants using MetaCore (Thomson Reuters, New York, NY) integrated software. Herein, we followed two different approaches: examination of biologic implication by associated variants; and examination of biologic implication by pooling together associated variants and differentially expressed genes. In the latter, three public available expression datasets from sarcoidosis (GEO accession numbers GSE18781 [28], GSE19314 [29], and GSE32887 [30]) were considered. For each Gene Expression Omnibus (GEO-GSE) dataset, we selected sarcoidosis cases and control subjects at a minimum 1:1 ratio (details are provided in Table E7 in the online supplement) and performed expression analysis using the online GEOR2 tool (<http://www.ncbi.nlm.nih.gov/geo/geo2r/>). Subsequently, results were downloaded and mapped to chromosome/position using the Affymetrix-Human-Genome-U133-Plus-2.0-Array annotation-data (chip_hgu133plus2) Bioconductor R-package (Affymetrix, Inc., Santa Clara, CA). After mapping, a subset of probe-IDs with false discovery rate less than 0.05 identified as differentially expressed was extracted and uploaded onto MetaCore.

Results

Association Findings

LS versus healthy control subjects. The association analysis on LS (345 cases vs. 2,025 control subjects) revealed rs3130288 (OR, 4.01; 95% CI, 3.26–4.95; $P_{GC} = 6.47 \times 10^{-34}$) located in the 3' UTR of *ATF6B* (also

known as *CREBL1*), which localizes in major histocompatibility complex (MHC) class III region as the most significant finding. A large number of high signals (1,246 SNPs at $P_{GC} < 5 \times 10^{-8}$) was identified spanning from the extended MHC class I to class II regions (from *LRRIC16*, chr6:25558005 to *HLA-DPB2*, chr6:33191175). The top 25 significant SNPs are provided in Table 1. The entire list of LS-associated SNPs can be found as Table E1. Outside the MHC region, rs13407913 (OR, 1.48; 95% CI, 1.25–1.74; $P_{GC} = 2.31 \times 10^{-5}$) located in an intron of *ADCY3* on chromosome 2 was observed. Manhattan plot of association statistics is shown in Figure 1A. A close-up association regional plot is shown in Figure 2. Genomic control showed small dispersion for LS with an inflation factor lambda of 1.16. Q-Q plot for the observed versus expected *P* values and distribution of significant SNPs (defined by $P_{GC} < 5 \times 10^{-8}$) by variant location are shown in Figures E1 and E2, respectively. Suggestive signals (692 SNPs at $5 \times 10^{-8} < P_{GC} < 5 \times 10^{-5}$) enriching the extended MHC region were also identified and are provided in Table E1.

Results after conditioning on the top signal rs3130288 (see Table E2) showed that residual associations in the MHC classical class II locus were predominant, as indicated by leading SNP rs4642516 ($P_{cond,GC} = 6.69 \times 10^{-7}$) located between *HLA-DQB1* and *HLA-DQA2*. Additionally, findings at $P_{cond,GC}$ less than 5×10^{-5} were also noted in non-MHC genomic loci, 55 SNPs located near *ERAP1*, in *ERAP2* and *LNPEP*, and near *LIX1*, all on chromosome 5, and one SNP between *CSMD1* and *MCPH1* on chromosome 8. The Manhattan and Q-Q plots after conditioning are shown in Figure E3. Regional association plots for LS-associated SNPs on chromosome 6 at P_{GC} less than 5×10^{-8} before and after conditioning on the top signal are illustrated in Figures E4A and E4B. Regional association plot for suggestive finding is shown in Figure E5.

Additionally, because *HLA-DRB1*03* plays a strong role in the course of the disease, which is particularly seen in patients with LS, we stratified our LS dataset (cases and control subjects) by *HLA-DRB1*03* carriers and noncarriers and performed Immunochip analysis on these subgroups. The association test in *HLA-DRB1*03* carriers (513 healthy control subjects and 253 LS) identified rs2248462

Table 1. Results of Immunochip Analysis on LS versus Healthy Control Subjects Highlighting the Top 25 SNPs at Significance P Less Than 5×10^{-8}

SNP	CHR	BP	Gene (Nearby Genes)	CA/NCA	CAF	Discovery Cohort (Sweden, European Ancestry) ($n = 2,470$)			Replicating Cohort (Germany, European Ancestry) ($n = 4,498$)		
						OR (95% CI)	P Value	P_{GC}	OR (95% CI)	P Value	
rs3130288	6	32203979	CREBL1	A/C	0.345	4.02 (3.26-4.95)	3.29E-39	6.47E-34	4.13 (2.83-6.02)	1.66E-15	
rs3129927	6	32441805	C6orf10	C/A	0.343	3.99 (3.24-4.92)	1.09E-38	1.82E-33	4.19 (2.87-6.11)	7.79E-16	
rs2187668	6	32713862	HLA-DQA1	A/G	0.359	3.97 (3.22-4.89)	1.80E-38	2.79E-33	3.84 (2.65-5.57)	2.93E-14	
rs2854275	6	32736406	HLA-DQB1	A/C	0.357	3.96 (3.21-4.88)	2.64E-38	3.87E-33	3.74 (2.57-5.44)	1.42E-13	
rs3129950	6	32466179	(C6orf10, BTNL2)	C/G	0.342	3.98 (3.22-4.91)	5.22E-38	6.96E-33	4.33 (2.98-6.31)	7.89E-17	
rs9268219	6	32392086	C6orf10	C/A	0.334	3.97 (3.22-4.90)	5.59E-38	7.38E-33	4.34 (2.98-6.33)	1.14E-16	
rs3129716	6	32765414	(HLA-DQB1, HLA-DQA2)	G/A	0.356	3.92 (3.19-4.83)	5.78E-38	7.60E-33	3.73 (2.57-5.43)	1.61E-13	
rs2856674	6	32767623	(HLA-DQB1, HLA-DQA2)	G/A	0.357	3.93 (3.19-4.84)	6.08E-38	7.93E-33	3.74 (2.57-5.44)	1.49E-13	
rs2395149	6	32433540	C6orf10	A/G	0.343	3.92 (3.19-4.83)	7.82E-38	9.85E-33	4.19 (2.87-6.11)	7.79E-16	
rs9268235	6	32398186	C6orf10	A/G	0.343	3.91 (3.18-4.82)	1.06E-37	1.28E-32	4.18 (2.86-6.09)	8.86E-16	
rs1265757	6	32410360	C6orf10	A/G	0.343	3.91 (3.18-4.82)	1.06E-37	1.28E-32	4.18 (2.87-6.10)	8.31E-16	
rs3135394	6	32516475	HLA-DRA	G/A	0.344	3.84 (3.13-4.72)	1.19E-37	1.41E-32	4.21 (2.89-6.14)	6.03E-16	
rs3130490	6	31847099	C6orf27	A/C	0.339	3.83 (3.12-4.70)	1.84E-37	2.05E-32	3.79 (2.58-5.56)	2.97E-13	
rs775397	6	32369230	C6orf10	C/A	0.343	3.87 (3.14-4.76)	2.45E-37	2.63E-32	4.18 (2.87-6.10)	8.42E-16	
rs9268177	6	32382860	C6orf10	A/C	0.342	3.87 (3.14-4.76)	2.56E-37	2.73E-32	4.18 (2.87-6.10)	8.31E-16	
rs389884	6	32048876	STK19	G/A	0.342	3.82 (3.11-4.69)	3.80E-37	3.84E-32	4.09 (2.80-5.96)	2.76E-15	
rs1150753	6	32167845	TNXB	G/A	0.341	3.83 (3.11-4.71)	4.27E-37	4.24E-32	4.12 (2.82-6.01)	1.88E-15	
rs3132971	6	32338234	(NOTCH4, C6orf10)	C/A	0.341	3.86 (3.13-4.75)	8.76E-37	7.86E-32	4.18 (2.86-6.09)	8.97E-16	
rs4143332	6	31456344	(HCP5, 3.8-1)	A/G	0.337	3.78 (3.07-4.65)	1.56E-36	1.29E-31	3.64 (2.50-5.31)	6.88E-13	
rs3131618	6	31542600	(APOM1, C6orf47)	G/A	0.340	3.81 (3.09-4.69)	2.40E-36	1.87E-31	3.72 (2.55-5.42)	2.69E-13	
rs3132449	6	31733992	(APOM1, C6orf47)	A/G	0.340	3.71 (3.03-4.55)	2.46E-36	1.91E-31	3.94 (2.70-5.75)	1.73E-14	
rs3117577	6	31835453	MSH5	G/A	0.339	3.70 (3.02-4.54)	2.85E-36	2.17E-31	3.96 (2.72-5.77)	1.36E-14	
rs519417	6	31986412	(ZBTB12, C2)	A/G	0.340	3.71 (3.03-4.56)	3.26E-36	2.43E-31	4.01 (2.75-5.85)	6.91E-15	
rs497309	6	32000463	(ZBTB12, C2)	C/A	0.340	3.71 (3.02-4.55)	3.79E-36	2.77E-31	4.03 (2.76-5.88)	5.71E-15	
rs1270942	6	32026839	CFB	G/A	0.339	3.72 (3.03-4.56)	4.06E-36	2.94E-31	4.05 (2.78-5.91)	4.43E-15	

Definition of abbreviations: BP = base pairs; CA = coded allele; CAF = coded allele frequency; CHR = chromosome; CI = confidence interval; LS = Lofgren's syndrome; NCA = noncoded allele; OR = odds ratio; P = unadjusted P value; P_{GC} = adjusted P value by genomic control ($\lambda_{GC} = 1.16$); SNP = single-nucleotide polymorphism. Genetic effects (OR) adjusted according to sex, coded allele. OR is the risk on LS (0/1) per allele based on the additive genetic model. 95% CI is the 95% CI for the OR. The entire list of LS-associated SNPs is provided in Table E2.

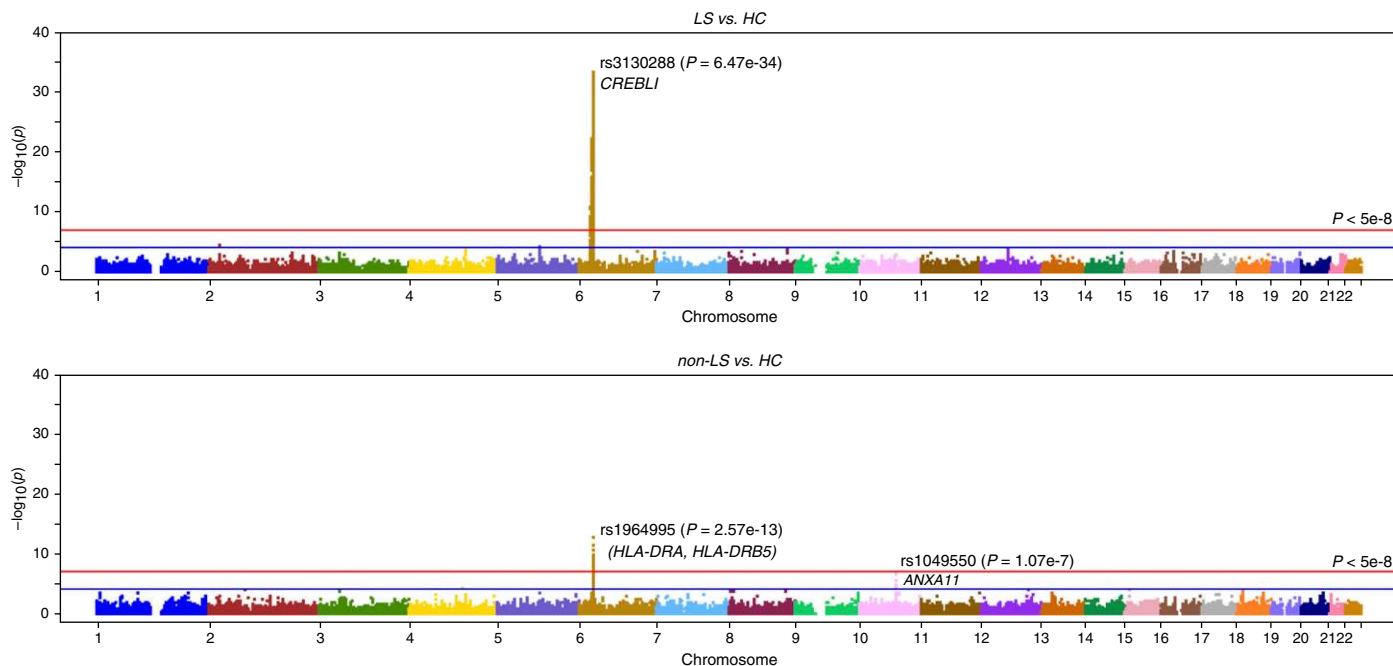


Figure 1. Manhattan plot of all markers from the Immunochip. Each dot represents the P_{GC} value of association analysis [$-\log_{10}(P_{GC})$] on the y-axis and position in chromosome on the x-axis (P_{GC} is genomic-controlled P value). (Top) Löfgren's syndrome (LS) versus control subjects and (bottom) non-LS versus control subjects. Red line indicates $P_{GC} < 5 \times 10^{-8}$; blue line is $P_{GC} < 5 \times 10^{-5}$. HC = healthy control subjects.

($P_{GC} = 1.20 \times 10^{-4}$), an intergenic SNP located *HCP5* and *MICB* on chr6:31554775 as the top signal. Similarly, in *HLA-DRB1*03* noncarriers (1,573 healthy control subjects and 131 LS), the test revealed rs3104407 ($P_{GC} = 1.25 \times 10^{-5}$), an intergenic SNP located between *HLA-DQB1* and *HLA-DQA2* on chr6:32790430 as the top signal (further details on these analyses and results are given in the online supplement NOTE). Notably, Immunochip results for *HLA-DRB1*03* stratified groups were consistent with Immunochip results for conditioning on *HLA-DRB1*03* (see the NOTE in the online supplement and Figure E6), namely all identified associations for LS substantially attenuate (with $P > 5 \times 10^{-8}$) when the effect of *HLA-DRB1*03* is considered.

Non-LS versus healthy control subjects.

Results from association analysis on non-LS (568 cases vs. 2,086 control subjects) showed rs1964995 (OR, 0.55; 95% CI, 0.47–0.64; $P_{GC} = 2.57 \times 10^{-13}$) located between *HLA-DRA* and *HLA-DRB5*, which localizes to MHC class II locus as the most significant signal. Fifty-two high signals located between *C6orf10* and *HLA-DQA2* (chr6:32447326–32789970) and between *PLAC9* and *ANXA11* (chr10:8191705–81916682) at $P_{GC} < 5 \times 10^{-8}$ were identified. The top 25 significant SNPs

are provided in Table 2. The entire list of non-LS-associated SNPs can be found in Table E3. Manhattan plot for Immunochip associations is illustrated in Figure 1B. A close-up association regional plot is shown in Figure 2. Genomic control for non-LS defined by inflation factor lambda was 1.17. The Q-Q plot and distribution of significant SNPs ($P_{GC} < 5 \times 10^{-8}$) by variant type are illustrated in Figures E7 and E8, respectively. Suggestive signals (122 SNPs included in Table E3) at P_{GC} greater than 5×10^{-8} and less than 5×10^{-5} revealed additional signals in the MHC region, particularly clustering between classes III and II regions and outside the extended MHC on chromosome 10 in *ANXA11* and near *PLAC9*, *DYDC2*, *C10orf58*, and *TSPAN14*.

Results after conditioning on top signal rs1964995 (see Table E4) stressed rs1049550 ($P_{cond,GC} = 2.19 \times 10^{-7}$) located in a coding region of *ANXA11* as the highest peak, followed by four intergenic SNPs, adjacent to previously observed genes. Suggestive signals were also observed, of which most (intergenic SNPs) were clustered together among the HLA class II genes (chr6:32411670–32873563). Manhattan and Q-Q plots after conditioning are shown in Figure E9. Regional association plots for non-LS-associated SNPs on chromosome 6 at P_{GC} less than 5×10^{-8}

before and after conditioning on the top signal are illustrated in Figures E10A and E10B. Regional association plot for suggestive signals at P_{GC} less than 5×10^{-5} is illustrated in Figure E11. Association results from evaluating both phenotypes, LS versus non-LS including conditioning on the top signal and on *HLA-DRB1* alleles, and replication results are provided in the NOTE in the online supplement.

Additionally, considering that the non-LS phenotype may be confounded by phenotype heterogeneity because of the presence of extrapulmonary manifestations as often seen in patients without LS, we reran Immunochip analysis by excluding patients without LS with confirmed extrapulmonary manifestations. Herein, 451 non-LS cases without extrapulmonary manifestations and 2,086 healthy control subjects were reanalyzed. Immunochip results showed rs1964995 (OR, 0.50; 95% CI, 0.42–0.59; $P_{GC} = 4.31 \times 10^{-13}$) as the top association along with similar high and suggested signals as identified in the main Immunochip for non-LS, thus implying that the same dominating gene associations are important in both non-LS groups with or without extrapulmonary manifestations. Results from Immunochip for non-LS without pulmonary manifestation are

Table 2. Results of Immunochip Analysis on Non-LS versus Healthy Control Subjects Highlighting the Top 25 SNPs at Significance P Less Than 5×10^{-8}

SNP	CHR	BP	Gene (Nearby Genes)	CA/NCA	CAF	Discovery Cohort (Sweden, European Ancestry) ($n = 2,750$)			Replicating Cohort (Germany, European Ancestry) ($n = 4,911$)			Replicating Cohort (African American Ancestry) ($n = 1,657$)		
						OR (95% CI)	P Value	P_{ac}	OR (95% CI)	P Value	OR (95% CI)	P Value	OR (95% CI)	P Value
rs1964995	6	32557389	(HLA-DRA, HLA-DRB5)	G/A	0.296	0.55 (0.47–0.64)	2.47E-15	2.57E-13	0.56 (0.48–0.66)	1.19E-12	0.70 (0.59–0.82)	1.95E-05		
rs9269110	6	32551247	(HLA-DRA, HLA-DRB5)	A/C	0.389	1.74 (1.51–2.01)	5.78E-14	3.82E-12	1.34 (1.16–1.56)	6.20E-05	—	—		
rs9269081	6	32549078	(HLA-DRA, HLA-DRB5)	A/C	0.376	1.71 (1.48–1.97)	4.01E-13	2.01E-11	1.33 (1.14–1.54)	1.63E-04	—	—		
rs6923504	6	32536164	(HLA-DRA, HLA-DRB5)	G/C	0.381	1.66 (1.44–1.91)	4.05E-12	1.46E-10	1.35 (1.16–1.56)	6.09E-05	—	—		
rs6903608	6	32536263	(HLA-DRA, HLA-DRB5)	G/A	0.381	1.66 (1.44–1.91)	4.05E-12	1.46E-10	1.36 (1.17–1.57)	3.55E-05	—	—		
rs9268882	6	32539601	(HLA-DRA, HLA-DRB5)	A/G	0.381	1.66 (1.44–1.91)	4.54E-12	1.61E-10	1.36 (1.17–1.57)	3.86E-05	—	—		
rs3998158	6	32789970	(HLA-DQB1, HLA-DQA2)	G/A	0.168	0.53 (0.45–0.64)	5.35E-12	1.85E-10	0.60 (0.49–0.74)	9.45E-07	1.19 (0.98–1.43)	7.47E-02		
rs9275580	6	32788048	(HLA-DQB1, HLA-DQA2)	A/G	0.172	0.53 (0.45–0.64)	5.56E-12	1.91E-10	0.59 (0.48–0.72)	2.42E-07	1.29 (1.08–1.54)	4.29E-03		
rs9275580	6	32787440	(HLA-DQB1, HLA-DQA2)	G/A	0.178	0.54 (0.46–0.65)	9.71E-12	3.08E-10	0.60 (0.50–0.74)	4.99E-07	1.42 (1.20–1.67)	3.98E-05		
rs2395153	6	32453573	(C6orf10, BTNL2)	C/G	0.273	0.59 (0.51–0.69)	1.46E-11	4.38E-10	0.68 (0.58–0.79)	8.61E-07	0.63 (0.53–0.75)	2.12E-07		
rs3129948	6	32462622	(C6orf10, BTNL2)	C/A	0.390	1.63 (1.41–1.88)	4.11E-11	1.06E-09	1.35 (1.17–1.56)	5.66E-05	1.35 (1.14–1.60)	6.34E-04		
rs3129954	6	32466491	(C6orf10, BTNL2)	G/A	0.390	1.59 (1.38–1.84)	2.37E-10	4.78E-09	1.35 (1.16–1.56)	6.45E-05	1.36 (1.14–1.61)	4.63E-04		
rs4424066	6	32462406	(C6orf10, BTNL2)	A/G	0.390	1.59 (1.38–1.84)	2.37E-10	4.78E-09	1.35 (1.16–1.56)	6.10E-05	1.34 (1.13–1.59)	8.91E-04		
rs3129961	6	32462406	(C6orf10, BTNL2)	G/A	0.314	0.62 (0.54–0.72)	2.54E-10	5.06E-09	0.70 (0.60–0.81)	3.09E-06	0.75 (0.64–0.87)	1.97E-04		
rs3129955	6	32473818	BTNL2	A/G	0.390	1.59 (1.38–1.84)	3.49E-10	6.64E-09	1.34 (1.16–1.55)	7.52E-05	1.35 (1.14–1.60)	4.18E-04		
rs4373382	6	32458846	(C6orf10, BTNL2)	C/A	0.313	0.63 (0.54–0.73)	4.16E-10	7.72E-09	0.69 (0.59–0.80)	1.58E-06	0.75 (0.65–0.87)	1.73E-04		
rs3817973	6	32469089	(C6orf10, BTNL2)	A/G	0.314	0.63 (0.54–0.73)	4.35E-10	8.02E-09	0.69 (0.59–0.80)	1.57E-06	0.75 (0.65–0.88)	2.68E-04		
rs2076529	6	32471933	BTNL2	A/G	0.314	0.63 (0.54–0.73)	4.35E-10	8.02E-09	0.69 (0.59–0.80)	1.48E-06	0.75 (0.65–0.88)	2.56E-04		
rs9268472	6	32463583	(C6orf10, BTNL2)	A/G	0.351	0.64 (0.56–0.74)	8.35E-10	1.40E-08	0.67 (0.58–0.78)	2.40E-07	—	—		
rs9271588	6	32698931	(HLA-DRB1, HLA-DQA1)	G/A	0.351	0.64 (0.56–0.74)	8.35E-10	1.40E-08	0.67 (0.58–0.78)	2.40E-07	—	—		
rs2858332	6	32789139	(HLA-DQB1, HLA-DQA2)	A/C	0.354	0.65 (0.56–0.75)	1.47E-09	2.28E-08	1.36 (1.18–1.57)	3.07E-05	—	—		
rs3830135	6	32656442	HLA-DRB1	A/G	0.054	0.42 (0.32–0.56)	1.82E-09	2.74E-08	0.56 (0.42–0.74)	3.16E-05	—	—		

Definition of abbreviations: BP = base pairs; CA = coded allele; CAF = coded allele frequency; CHR = chromosome; CI = confidence interval; LS = Löffgren's syndrome; NCA = noncoded allele; OR = odds ratio; P = unadjusted P value; P_{ac} = adjusted P value by genomic control ($\lambda_{\text{mbda}} = 1.17$); SNP = single-nucleotide polymorphism. Genetic effects (OR) adjusted according to sex, coded allele. OR is the risk on LS (0/1) per allele based on the additive genetic model. 95% CI is the 95% CI for the OR. The entire list of non-LS-associated SNPs is provided in Table E3.

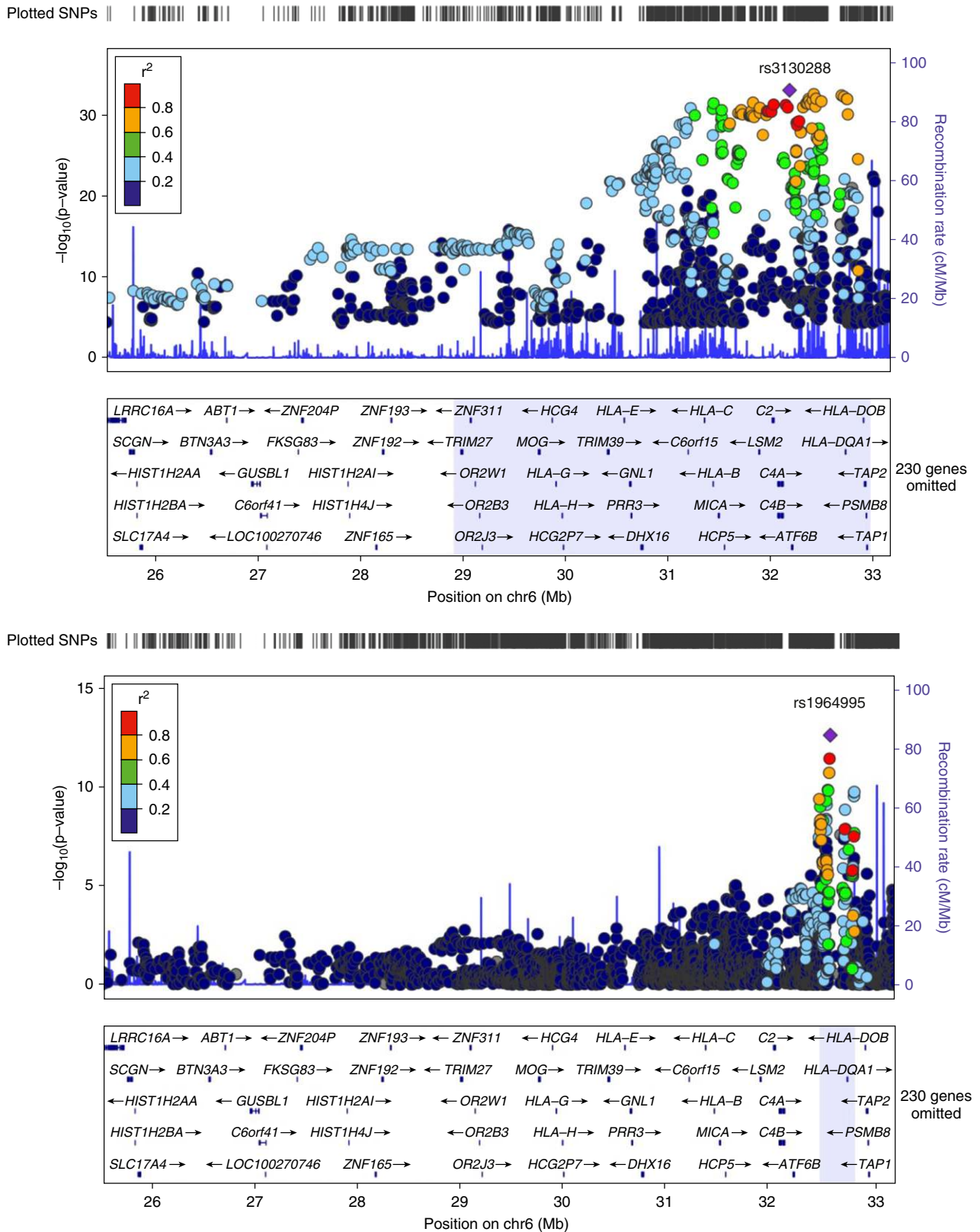


Figure 2. Regional association plots of chr6:25527073–33191175. Löfgren’s syndrome (LS)-associated single-nucleotide polymorphisms (SNPs) (top) at $P_{GC} < 5 \times 10^{-8}$ highlight region chr6:28910128–3295881. Non-LS-associated SNPs (bottom) at $P_{GC} < 5 \times 10^{-8}$ highlight region chr6:32447326–32789970. P_{GC} = genomic-controlled P value.

adjoined with main Immunochip findings for non-LS as given in Table E3.

Replication of Significant SNPs

Results from the association analysis on LS (64 LS vs. 4,498 control subjects) conducted in an independent German cohort confirmed the association of many signals. In the LS group, out of 1,246 high signals identified in the discovery cohort, 671 were confirmed with Bonferroni-adjusted P less than 4×10^{-5} (0.05/1,246) to account for multiple testing. The top signal rs3130288 identified in the discovery cohort was well replicated (Germany: OR, 4.13; 95% CI, 2.83–6.02; $P = 1.66 \times 10^{-15}$) (the Netherlands: OR, 4.54; 95% CI, 2.97–6.93; $P = 2.82 \times 10^{-12}$) (Czech Republic: OR, 4.94; 95% CI, 2.53–9.65; $P = 2.88 \times 10^{-6}$). Confirmed signals localized to the extended MHC region spanning from *ZNF184* to *HLA-DPB2* (chr6:27521903–33191175), as illustrated in the regional association plot (Figure 2). Replication for assessing 692 suggestive signals identified with P_{GC} greater than 5×10^{-8} and less than 5×10^{-5} in the Swedish population resulted in the confirmation of 56 suggested signals with Bonferroni-adjusted P less than 7.22×10^{-5} (0.05/692), which localized to MHC class I and class II regions. Non-MHC suggested signals located in *LRRC16A* (rs6921589, $P = 6.54 \times 10^{-2}$ and rs9295661, $P = 6.61 \times 10^{-2}$) were borderline significant. Signals in *ADCY3* (rs13407913, rs6752378, and rs10182181) were not confirmed in the German cohort, possibly because of few number of LS cases. Results from this analysis are given in Table E1.

In the non-LS group, out of 38 high signals identified in the discovery cohort, 27 were confirmed in the German cohort and 20 in the African American cohort with Bonferroni-adjusted P less than 1.32×10^{-3} (0.05/38). The lead signal rs1964995 was well replicated in all participating cohorts (Germany: OR, 0.56; 95% CI, 0.49–0.66; $P = 1.19 \times 10^{-12}$) (African American: OR, 0.70; 95% CI, 0.59–0.82; $P = 1.95 \times 10^{-5}$) (the Netherlands: OR, 0.71; 95% CI, 0.51–0.97; $P = 3.31 \times 10^{-2}$) (Czech Republic: OR, 0.55; 95% CI, 0.39–0.77; $P = 4.05 \times 10^{-4}$). All confirmed signals localized to MHC class II region, clustering among *C6orf10*, *BTNL2*, *HLA-DRA*, *HLA-DRB5*, *HLA-DRB1*, *HLA-DQA1*, and *HLA-DQA2*, as illustrated in the regional association plot (Figure 1). Replication assessment of 135 suggestive signals confirmed 41 suggestive signals in the Germany cohort and 30 suggested signals in

the USA-AA cohort with Bonferroni-adjusted P less than 3.70×10^{-4} (0.05/135). These localized in the same regions as confirmed high signals, thus enriching the density of the associated region. Out of the seven signals located outside the MHC, three signals (rs1953600, rs2573346, and rs1049550; in strong LD, $R^2 \geq 0.87$, $D' \geq 0.93$) located between *PLAC9* and *ANXA11* (intergenic), and inside *ANXA11* (intronic and coding) were replicated with nominal

significance P less than 0.05. Results from this analysis are given in Table E3.

Results from evaluating the association of six SNPs located outside the MHC region and conducted in two independent cohorts from Czech Republic and the Netherlands led to the confirmation of two SNPs. For LS, rs10108612 (discovery $P_{GC} = 1.97 \times 10^{-2}$) located between *CSMD1* and *MCPH1* on chromosome 8 was marginally significant ($P = 5.81 \times 10^{-2}$) in

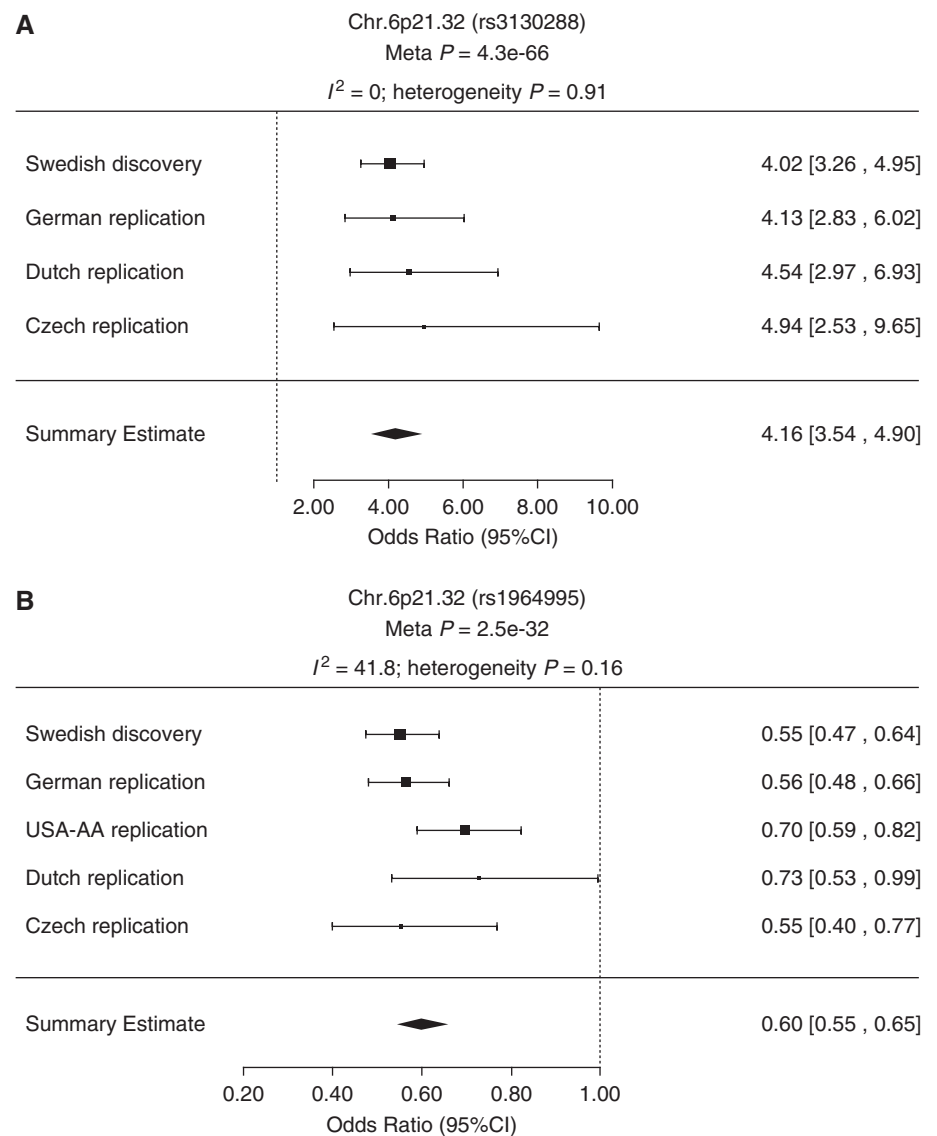


Figure 3. Forest plots showing Lögren's syndrome (LS) and non-LS lead single-nucleotide polymorphism (SNP) associations inside the major histocompatibility complex. Plots show the study-specific association estimates (odds ratio) and 95% confidence intervals (CIs) for discovery set (Swedish) and all replication studies (German, African American [USA-AA], Dutch, and Czech). Rs3130288 (A) and rs1964995 (B) are the lead SNPs associated with LS and non-LS, respectively. The summary estimate includes the association estimate and CI under fixed-effect model for the meta-analysis combining the discovery and replicating cohort shown as a diamond. I^2 indicates the percentage of total variation in study estimates because of heterogeneity in the meta-analysis.

the Czech cohort with and nonsignificant in the Dutch cohort. Similarly, rs13407913 (discovery $P_{GC} = 2.31 \times 10^{-5}$) located in an intron of *ADCY3* on chromosome 2 was marginally significant ($P = 7.8 \times 10^{-2}$) in the Dutch LS group and nonsignificant in the Czech LS group. For non-LS, no associations among the tested SNPs were found in the either Czech or Dutch samples.

In summary, combining replicated high and suggestive signals, we observed that 710 confirmed variants were associated exclusively to LS compared with control subjects, 51 to non-LS compared with control subjects, and 17 were associated to both LS and non-LS. Whereas in a population of African ancestry, out of 50 signals associated with non-LS, 17 were LS-associated signals found in populations of European ancestry. In populations with European ancestry, the shared genomic loci associated to both LS and non-LS spanned between two intergenic regions between *C6orf10* and *BTNL2* and

between *HLA-DRA* and *HLA-DRB5* (chr6:32453573–32528157), whereas in a population with African ancestry it extended along chromosome 6 to an intergenic region located between *HLA-DQB1* and *HLA-DQA2* (chr6:32453573–32778286).

Meta-analysis of Associated SNPs

In the meta-analysis, results for estimating the overall effect size (OR) of lead SNPs associated with LS and non-LS, respectively, were highly significant (rs3130288 meta- $P = 4.3 \times 10^{-66}$ and rs1964995 meta- $P = 2.5 \times 10^{-32}$) (Figure 3). Likewise, results for SNPs located outside the MHC region showed a similar pattern: for LS, rs13407913 (meta- $P = 3.8 \times 10^{-3}$), rs9295661 (meta- $P = 8.1 \times 10^{-10}$), and rs10108612 (meta- $P = 2.3 \times 10^{-2}$); and for non-LS, rs1953600 (meta- $P = 1.3 \times 10^{-11}$), rs2573346 (meta- $P = 1.9 \times 10^{-10}$), and rs1049550 (meta- $P = 2.6 \times 10^{-11}$). Forest

plots for SNPs outside the MHC are illustrated in Figure 4.

Combining association summary statistics from both Swedish and German cohorts, results from meta-analysis on 1,938 high and suggested LS signals revealed 1,499 signals (defining 267 loci; 22/1,499 signals had heterogeneity $P < 0.05$ and $I^2 = 0\%$) under the fixed-effects model and 1,320 signals (defining 241 loci; 116/1,320 had $I^2 = [0.7\%, 62.4\%]$) under the random-effects model with strong evidence for association at meta- P less than 5×10^{-8} . Table 3 provides the top 25 LS-associated findings. The entire list for LS-associated SNPs can be found in Table E5. Similarly, meta-analysis combining association summary statistics from Swedish, German, and African American cohorts for evaluating 173 high and suggested non-LS signals showed 47 signals (defining 12 loci) with strong evidence for association under the fixed-effects model and 37 signals (defining eight loci) under the random-effects model,

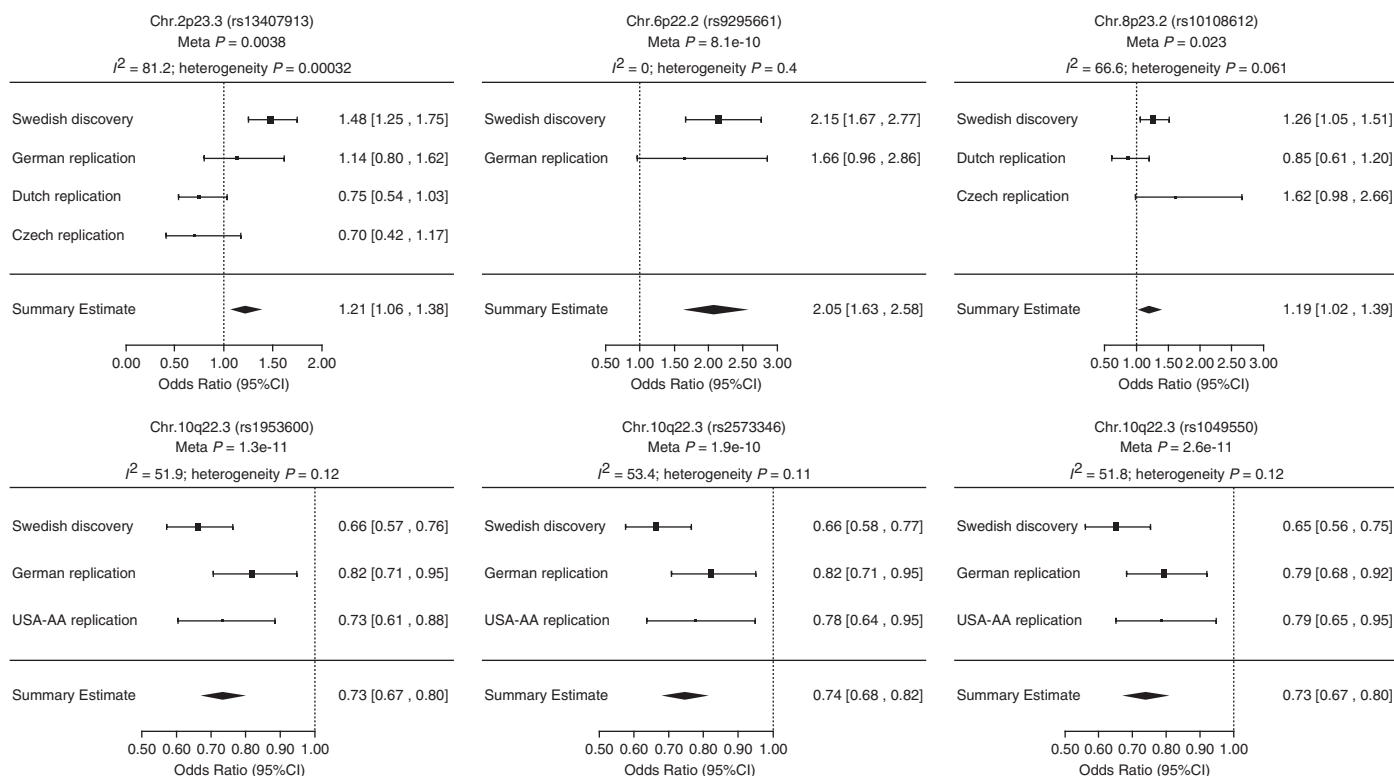


Figure 4. Forest plots showing Lögren's syndrome (LS) and non-LS single-nucleotide polymorphism (SNP) associations outside the major histocompatibility complex. Plots show the study-specific association estimates (odds ratio) and 95% confidence intervals (CIs) for discovery set (Swedish) and replication studies (German, African American [USA-AA], Dutch, Czech). Results for LS-associated SNPs rs13409913, rs9295661, and rs10108612 are shown in the top. Results for non-LS-associated SNP rs1953600, rs2573346, and rs1049550 are shown in the bottom. The association estimate and CI (estimated under fixed-effect model) for the meta-analysis combining the discovery and replicating cohort are shown as a diamond. Blank spaces indicate occasions in which a particularly study was not able to provide results for a given SNP. I^2 indicates the percentage of total variation in study estimates because of heterogeneity in the meta-analysis.

Table 3. Results from Combined Meta-analysis on LS-associated SNPs at Significance Meta-P Less Than 5×10^{-8} (Top 25 Findings)

SNP	CHR	BP	CA/NCA	Gene (Nearby Genes)	SNP Location	Cohorts	Fixed-Effects Model		Random-Effects Model	
							OR (95% CI)	Meta-P Value	OR (95% CI)	Meta-P Value
rs3130288	6	32203979	A/C	CREBL1	3' UTR	SWE	4.16 (3.54-4.90)	4.30E-66	4.16 (3.54-4.90)	4.30E-66
rs3129927	6	32441805	C/A	C6orf10	Intron	GER, SWE	4.04 (3.36-4.84)	1.29E-50	4.04 (3.36-4.84)	1.29E-50
rs9268219	6	32392086	C/A	C6orf10	Intron	GER, SWE	4.05 (3.37-4.87)	1.51E-50	4.05 (3.37-4.87)	1.51E-50
rs3129950	6	32466179	C/G	(C6orf10, BTNL2)	Intergenic	GER, SWE	4.06 (3.38-4.88)	1.79E-50	4.06 (3.38-4.88)	1.79E-50
rs2395149	6	32433540	A/G	C6orf10	Intron	GER, SWE	3.98 (3.32-4.77)	3.55E-50	3.98 (3.32-4.77)	3.55E-50
rs3135394	6	32516475	G/A	HLA-DRA	Intron	GER, SWE	3.92 (3.27-4.70)	7.66E-50	3.92 (3.27-4.70)	7.66E-50
rs1265757	6	32410360	A/G	C6orf10	Intron	GER, SWE	3.97 (3.31-4.77)	8.02E-50	3.97 (3.31-4.77)	8.02E-50
rs9268235	6	32398186	A/G	C6orf10	Intron	GER, SWE	3.97 (3.31-4.76)	8.22E-50	3.97 (3.31-4.76)	8.22E-50
rs775397	6	32369230	C/A	C6orf10	Coding	GER, SWE	3.94 (3.28-4.73)	2.94E-49	3.94 (3.28-4.73)	2.94E-49
rs9268177	6	32382860	A/C	C6orf10	Intron	GER, SWE	3.94 (3.28-4.73)	2.98E-49	3.94 (3.28-4.73)	2.98E-49
rs2187668	6	32713862	A/G	HLA-DQA1	Intron	GER, SWE	3.94 (3.28-4.73)	3.29E-49	3.94 (3.28-4.73)	3.29E-49
rs389884	6	32048876	G/A	STK19	Intron	GER, SWE	3.88 (3.24-4.65)	4.28E-49	3.88 (3.24-4.65)	4.28E-49
rs3132971	6	32338234	C/A	(NOTCH4, C6orf10)	Intergenic	GER, SWE	3.93 (3.28-4.72)	6.55E-49	3.93 (3.28-4.72)	6.55E-49
rs1794282	6	32774504	A/G	(HLA-DQB1, HLA-DQA2)	Intergenic	GER, SWE	3.92 (3.26-4.70)	9.82E-49	3.92 (3.26-4.70)	9.82E-49
rs1150753	6	32167845	G/A	TNXB	Intron	GER, SWE	3.89 (3.25-4.67)	1.26E-48	3.89 (3.25-4.67)	1.26E-48
rs3129716	6	32765414	G/A	(HLA-DQB1, HLA-DQA2)	Intergenic	GER, SWE	3.88 (3.23-4.65)	1.77E-48	3.88 (3.23-4.65)	1.77E-48
rs2854275	6	32736406	A/C	HLA-DQB1	Intron	GER, SWE	3.91 (3.25-4.69)	2.39E-48	3.91 (3.25-4.69)	2.39E-48
rs2856674	6	32767623	G/A	(HLA-DQB1, HLA-DQA2)	Intergenic	GER, SWE	3.88 (3.24-4.66)	2.80E-48	3.88 (3.24-4.66)	2.80E-48
rs3130490	6	31847099	A/C	C6orf27	Intron	GER, SWE	3.82 (3.19-4.58)	6.92E-48	3.82 (3.19-4.58)	6.92E-48
rs1270942	6	32026839	G/A	CFB	Intron	GER, SWE	3.79 (3.17-4.54)	7.12E-48	3.79 (3.17-4.54)	7.12E-48
rs3132449	6	31733992	A/G	(APOM, C6orf47)	Intergenic	GER, SWE	3.76 (3.14-4.50)	1.11E-47	3.76 (3.14-4.50)	1.11E-47
rs519417	6	31986412	A/G	(ZBTB12, C2)	Intergenic	GER, SWE	3.78 (3.16-4.52)	1.34E-47	3.78 (3.16-4.52)	1.34E-47
rs497309	6	32000463	C/A	(ZBTB12, C2)	Intergenic	GER, SWE	3.78 (3.16-4.53)	1.82E-47	3.78 (3.16-4.53)	1.82E-47
rs3117577	6	31835453	G/A	MSH5	Intron	GER, SWE	3.76 (3.14-4.49)	1.96E-47	3.76 (3.14-4.49)	1.96E-47
rs3117582	6	31728499	C/A	(BAT3, APOM)	Intergenic	GER, SWE	3.73 (3.12-4.46)	2.53E-47	3.73 (3.12-4.46)	2.53E-47

Definition of abbreviations: BP = base pairs; CA = coded allele; CHR = chromosome; CI = confidence interval; GER = Germany; LS = Löffgren's syndrome; NCA = noncoded allele; OR = odds ratio; SNP = single-nucleotide polymorphism; SWE = Sweden; UTR = untranslated region. The entire list of LS-associated SNPs is provided in Table E5.

The overall association estimates (OR) and 95% CI for LS-associated SNPs under fixed-effect and random-effect models, respectively. Cohorts from SWE and GER are of European ancestry.

Table 4. Results from Combined Meta-analysis on Non-LS-associated SNPs at Significance Meta-P Less Than 5×10^{-8} (Top 25 Findings)

SNP	CHR	BP	CA/NCA	Gene (Nearby Genes)	SNP Location	Cohorts	Fixed-Effects Model			Random-Effects Model			I ² P Value	f ² (%)
							OR (95% CI)	P Value	Meta-P Value	OR (95% CI)	P Value	Meta-P Value		
rs1964995	6	32557389	G/A	(HLA-DRA, HLA-DRB5)	Intergenic	SWE, GER, USA-AA	0.60 (0.55-0.65)	2.53E-32	5.99E-17	0.61 (0.54-0.68)	1.55E-01	41.79		
rs2213585	6	32521128	G/A	(HLA-DRA, HLA-DRB5)	Intergenic	SWE, GER, USA-AA	1.51 (1.39-1.63)	8.82E-23	3.36E-11	1.51 (1.34-1.70)	1.11E-01	54.69		
rs2227139	6	32521437	G/A	(HLA-DRA, HLA-DRB5)	Intergenic	SWE, GER, USA-AA	1.50 (1.39-1.63)	8.96E-23	6.87E-11	1.51 (1.33-1.70)	1.03E-01	56.15		
rs2213586	6	32521072	A/G	(HLA-DRA, HLA-DRB5)	Intergenic	SWE, GER, USA-AA	1.50 (1.38-1.63)	1.29E-22	4.58E-11	1.50 (1.33-1.70)	1.09E-01	54.98		
rs7192	6	32519624	A/C	HLA-DRA	Coding	SWE, GER, USA-AA	1.50 (1.39-1.63)	1.31E-22	8.78E-12	1.51 (1.34-1.69)	1.27E-01	51.57		
rs2395153	6	32453573	C/G	(C6orf10, BTNL2)	Intergenic	SWE, GER, USA-AA	0.63 (0.58-0.69)	1.76E-22	1.76E-22	0.63 (0.58-0.69)	5.17E-01	0.00		
rs7195	6	32520517	A/G	HLA-DRA	UTR	SWE, GER, USA-AA	1.50 (1.38-1.63)	1.77E-22	9.57E-13	1.50 (1.34-1.68)	1.52E-01	46.70		
rs3763327	6	32521808	G/C	(HLA-DRA, HLA-DRB5)	Intergenic	SWE, USA-AA	1.57 (1.42-1.73)	7.36E-19	2.75E-08	1.57 (1.34-1.85)	1.08E-01	61.20		
rs477515	6	32677669	A/G	(HLA-DRB1, HLA-DQA1)	Intergenic	SWE, GER, USA-AA	0.64 (0.58-0.71)	3.93E-18	5.32E-08	0.63 (0.54-0.75)	7.79E-02	61.89		
rs2395182	6	32521295	C/A	(HLA-DRA, HLA-DRB5)	Intergenic	SWE, GER, USA-AA	1.49 (1.36-1.62)	7.05E-18	4.95E-05	1.47 (1.22-1.78)	1.48E-02	76.86		
rs4373382	6	32458846	C/A	(C6orf10, BTNL2)	Intergenic	SWE, GER, USA-AA	0.69 (0.63-0.75)	1.17E-17	5.81E-13	0.69 (0.62-0.76)	2.48E-01	28.87		
rs3129952	6	32467741	C/G	(C6orf10, BTNL2)	Intergenic	SWE, USA-AA	1.64 (1.46-1.84)	1.54E-17	1.54E-17	1.64 (1.46-1.84)	4.18E-01	0.00		
rs4424066	6	32462406	G/A	BTNL2	Intergenic	SWE, GER, USA-AA	0.69 (0.63-0.75)	1.73E-17	3.44E-12	0.69 (0.62-0.76)	2.28E-01	32.82		
rs2076529	6	32471933	G/A	BTNL2	Coding	SWE, GER, USA-AA	0.69 (0.63-0.75)	1.74E-17	1.70E-12	0.69 (0.62-0.76)	2.37E-01	30.92		
rs3817973	6	32469089	A/G	(C6orf10, BTNL2)	Intergenic	SWE, GER, USA-AA	0.69 (0.63-0.75)	1.96E-17	2.39E-12	0.69 (0.62-0.76)	2.34E-01	31.62		
rs2239802	6	32519824	C/G	HLA-DRA	Intron	SWE, GER, USA-AA	1.48 (1.35-1.61)	1.97E-17	5.85E-05	1.46 (1.22-1.77)	1.56E-02	76.65		
rs9268472	6	32463583	A/G	(C6orf10, BTNL2)	Intergenic	SWE, GER, USA-AA	0.69 (0.63-0.75)	3.84E-17	3.06E-11	0.69 (0.62-0.77)	2.04E-01	37.34		
rs3177928	6	32520413	A/G	HLA-DRA	UTR	SWE, GER, USA-AA	0.53 (0.46-0.62)	8.37E-17	8.37E-17	0.53 (0.46-0.62)	6.77E-01	0.00		
rs2076530	6	32471794	G/A	BTNL2	Coding	SWE, GER, USA-AA	0.69 (0.64-0.76)	1.21E-16	1.21E-16	0.69 (0.64-0.76)	4.96E-01	0.00		
rs268969	6	32542327	A/G	(HLA-DRA, HLA-DRB5)	Intergenic	SWE, GER, USA-AA	0.66 (0.60-0.73)	1.29E-16	6.60E-16	0.66 (0.60-0.73)	6.60E-01	0.00		
rs3129888	6	32519704	G/A	HLA-DRA	Intron	SWE, GER, USA-AA	1.48 (1.35-1.62)	2.07E-16	6.22E-06	1.47 (1.25-1.74)	4.00E-02	68.94		
rs3129948	6	32462622	C/A	(C6orf10, BTNL2)	Intergenic	SWE, GER, USA-AA	1.44 (1.32-1.58)	2.30E-16	1.46E-08	1.44 (1.27-1.63)	1.31E-01	50.83		
rs2294878	6	32475773	A/C	BTNL2	Intron	SWE, GER, USA-AA	0.70 (0.64-0.76)	3.66E-16	3.66E-16	0.70 (0.64-0.76)	9.24E-01	0.00		
rs3117098	6	32466491	G/A	(C6orf10, BTNL2)	Intergenic	SWE, GER, USA-AA	1.44 (1.32-1.57)	6.96E-16	4.11E-10	1.43 (1.28-1.60)	2.01E-01	38.74		
rs3129961	6	32486918	G/A	(BTNL2, HLA-DRA)	Intergenic	SWE, GER, USA-AA	1.43 (1.31-1.56)	1.02E-15	6.67E-10	1.43 (1.28-1.60)	1.95E-01	39.66		

Definition of abbreviations: BP = base pairs; CA = coded allele; CHR = chromosome; CI = confidence interval; GER = Germany; LS = L6fren's syndrome; NCA = noncoded allele; OR = odds ratio; SNP = single-nucleotide polymorphism; SWE = Sweden; USA-AA = US African American; UTR = untranslated region.

The entire list of non-LS-associated SNPs is provided in Table E6. The overall association estimates (OR) and 95% CI for non-LS-associated SNPs under fixed-effect and random-effect models, respectively. The τ^2 P represents the P value significance for the amount of heterogeneity in the true log odds; the f^2 statistic represents how much of the total variability in the effect size estimate can be attributed to heterogeneity among the true effects. Cohorts from SWE and GER are of European ancestry; the USA-AA cohort is of African ancestry.

with strong evidence for association at meta- P less than 5×10^{-8} . Non-LS signals were selected with heterogeneity P greater than 0.05. Table 4 provides the 25 top non-LS-associated signals. The entire list for non-LS-associated SNPs can be found in Table E6.

Gene-centric Analysis

A gene-based approach identified five genomic loci (*BTNL2*, *HLA-DRA*, and *HLA-DRB1* on chr6, and *PLAC9* and *ANXA11* on chr10) associated with non-LS and 179 genomic loci (from *SCGN* to *HLA-DPB1* localizing to extended MHC on chr6) associated with LS as exceeding a Bonferroni-corrected threshold of P less than 7.3×10^{-6} . Remarkably, significant gene associations for both LS and non-LS identified by this method were similar to those identified by associated SNPs from ImmunoChip analyses, and

thus strengthened our findings. Table 5 lists the 15 most significant LS- and non-LS-associated genes obtained from VEGAS. Results for LS and non-LS at nominal P less than 0.05 are provided in Tables E7 and E8, respectively.

Integrative Analyses on Significant Findings

Bioinformatic analysis for refinement of associated variants. Results from studying the potential influence of confirmed variants on gene expression levels in various tissues and cell types revealed 564 of 710 LS-associated SNPs, 51 of 68 non-LS-associated SNPs, and 16 of 17 mutually associated SNPs as *cis*-eQTL SNPs (Figure 5).

Advanced query on unclassified SNPs using extended LD information uncovered 105 LS-associated SNPs and 12 non-LS-associated SNPs positioned near a *cis*-eQTL

SNP located on the same haplotype and in high LD ($R^2 > 0.8$). Moreover, 41 LS-associated SNPs and three non-LS-associated SNPs were found with no *cis*-eQTL mapping.

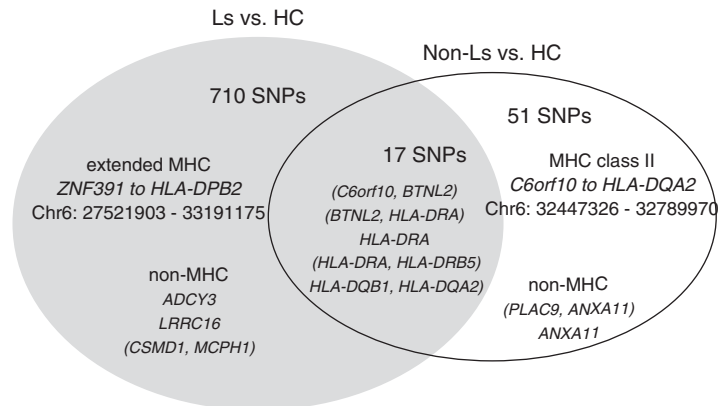
Results from assessing confirmed variants in elements of transcription regulation using datasets from ENCODE and the National Institutes of Health Roadmap Epigenomics Mapping Consortium identified 675 LS-associated SNPs, 49 non-LS-associated SNPs, and all 17 mutually SNPs as regulatory SNPs. A distribution diagram of confirmed variants by transcription-element classification is illustrated in Figure 5.

Enrichment and pathway analyses of associated variants. Results from enrichment analysis based on 727 LS-associated confirmed variants at false discovery rate less than 0.05 identified pathway maps related to immune response of

Table 5. VEGAS Results for the 15 Most Significant Genes from ImmunoChip Analysis on LS and non-LS (48,740 SNPs and 6,840 Genes)

CHR	Gene	nSNPs	Start Position	Stop Position	Test Statistic	P Value	Best SNP	SNP P Value
LS vs. HC								
6	<i>TNXB</i>	50	32116910	32185129	1206.77	1.00E-06	rs3130288	6.47E-34
6	<i>CREBL1</i>	51	32191022	32203995	1409.21	1.00E-06	rs3130288	6.47E-34
6	<i>FKBP1</i>	49	32204461	32206045	1464.31	1.00E-06	rs3130288	6.47E-34
6	<i>PRRT1</i>	61	32224117	32227698	2290.62	1.00E-06	rs3130288	6.47E-34
6	<i>PPT2</i>	68	32229278	32239430	2779.84	1.00E-06	rs3130288	6.47E-34
6	<i>EGFL8</i>	69	32240382	32244040	2728.04	1.00E-06	rs3130288	6.47E-34
6	<i>AGPAT1</i>	84	32243966	32253820	3085.42	1.00E-06	rs3130288	6.47E-34
6	<i>C6orf10</i>	149	32368452	32447634	5678.59	1.00E-06	rs3129927	1.82E-33
6	<i>BTNL2</i>	109	32470490	32482878	3946.51	1.00E-06	rs3129927	1.82E-33
6	<i>HLA-DRB1</i>	2	32654524	32665540	144.87	1.00E-06	rs2187668	2.79E-33
6	<i>HLA-DQA1</i>	2	32713160	32719407	288.31	1.00E-06	rs2187668	2.79E-33
6	<i>HLA-DQB1</i>	2	32735634	32742444	288.31	1.00E-06	rs2187668	2.79E-33
6	<i>HLA-DRA</i>	61	32515624	32520802	2278.93	1.00E-06	rs3135394	1.41E-32
6	<i>C2</i>	50	32003472	32021427	1027.18	1.00E-06	rs389884	3.84E-32
6	<i>CFB</i>	45	32021699	32027840	831.90	1.00E-06	rs389884	3.84E-32
Non-LS vs. HC								
6	<i>HLA-DRA</i>	61	32515624	32520802	671.61	1.00E-06	rs3129882	5.33E-10
6	<i>BTNL2</i>	109	32470490	32482878	935.64	1.00E-06	rs3129882	5.33E-10
6	<i>HLA-DRB1</i>	2	32654524	32665540	36.76	1.00E-06	rs3830135	2.74E-08
10	<i>ANXA11</i>	6	81904859	81955308	82.55	1.00E-06	rs1049550	1.07E-07
10	<i>PLAC9</i>	3	81882237	81894764	60.30	1.00E-06	rs1049550	1.07E-07
6	<i>HLA-DPB1</i>	58	33151737	33162954	363.18	9.40E-05	rs9277357	1.61E-05
18	<i>PTPN2</i>	54	12775479	12874334	317.12	9.70E-05	rs2852151	1.55E-04
6	<i>HLA-DPA1</i>	46	33140771	33149356	326.14	1.28E-04	rs9277357	1.61E-05
12	<i>PAH</i>	2	1.02E+08	1.02E+08	18.94	1.85E-04	rs2242381	1.10E-04
6	<i>LST1</i>	61	31661949	31664665	249.78	2.11E-04	rs2239704	1.53E-05
6	<i>TNF</i>	60	31651328	31654091	297.74	2.92E-04	rs2239704	1.53E-05
6	<i>LTB</i>	61	31656314	31658181	269.43	3.19E-04	rs2239704	1.53E-05
6	<i>NCR3</i>	60	31664650	31668741	225.25	3.71E-04	rs2239704	1.53E-05
6	<i>PSMB9</i>	87	32929915	32935606	357.37	4.05E-04	rs3819717	2.43E-06
6	<i>PSMB8</i>	87	32916471	32920690	357.37	4.25E-04	rs3819717	2.43E-06

Definition of abbreviations: CHR = chromosome; HC = healthy control subjects; LS = Löfgren's syndrome; nSNPs = number of SNPs included within a gene; SNP = single-nucleotide polymorphism; VEGAS = Versatile Gene-based Association Study. The entire lists of LS and non-LS genic SNPs at nominal $P < 0.05$ are provided in Tables E7 and E8, respectively.



	SNPs associated with LS	SNPs associated with LS and non-LS	SNPs associated with non-LS
SNPs found as <i>cis</i> -eQTL-SNP	564 (79.4%)	16 (94.1%)	38 (74.5%)
SNPs found nearby a <i>cis</i> -eQTL-SNP	105 (14.8%)	-	12 (92.3%)
SNPs found as regulatory-SNP	675 (95.1%)	17 (100%)	49 (96.1%)
- located in promoters	109 (15.4%)	2 (11%)	6 (11.8%)
- located in enhancers	261 (36.7%)	2 (11%)	13 (25.5%)
- located in DNase hypersensitivity	250 (35.2%)	5 (29.4%)	13 (25.5%)
- located in protein binding sites	152 (21.4%)	5 (29.4%)	7 (13.7%)
- located in TF binding sites	576 (81.1%)	16 (94.1%)	44 (86.3%)

Figure 5. Distribution of *cis*-expression quantitative trait loci (eQTL) and regulatory single-nucleotide polymorphisms (SNPs) among associated confirmed variants of Löfgren's syndrome (LS), non-LS, and both. Percentages are derived from the total number in each group, respectively. Genes enclosed in *parentheses* denote intergenic regions. HC = healthy control subjects; MHC = major histocompatibility complex; TF = transcription factor.

T-cell regulation and processes, and cytoskeleton remodeling. Pooling together 727 LS-associated SNPs and differentially expressed genes (from each GSE dataset tested) yielded similar pathway maps (see Table E9). In non-LS, assessment based on the 68 confirmed variants yielded no pathway maps. However, when combined with differentially expressed genes, identification of pathway maps related to immune response, differentiation, and regulation of T cells (see Table E10) was detected.

Noteworthy, many of the pathway maps that were identified in LS and non-LS, respectively, were overlapping by sharing genes with other pathways, and in some cases were subsets of each other. For example, in LS, the pathway maps labeled as a, b, c, d, f, g, i, and k (see Table E9) shared three common components: MHC class II, nuclear factor- κ B, and CD86. Likewise, in non-LS, pathway maps labeled 1, 2, 4, 6, 8, 11, 13, and 14 (see Table E10) shared one common

component: PCL-gamma 1. Further analysis on enrichment results from both phenotypes pinpointed the differences and shared pathway maps among these. Explicitly, pathway maps b-c-e-g-h (see Table E9) were unique to LS; pathway maps 1-2-3-4-6-7-8-9-11-12-13-14 (see Table E10) were unique to non-LS; and pathway maps a-10, c-17, d-5, i-16, k-15 were common to both. In the latter, the nuclear factor- κ B was unanimously present across the shared pathway maps.

Discussion

In this study, we report for the first time results of Immunochip analysis of LS and non-LS segregated as two different sarcoidosis entities in well-phenotyped cohorts with replicated and meta-analysis results. In this work, we aimed to investigate the genetic contributions to LS and non-LS by using the custom Illumina

Infinium array that leveraged the remarkable genetic overlap of 186 loci associated with autoimmunity. Results from various approaches in LS (using populations of European ancestry) and non-LS (using populations of European and African ancestry) show that genetic susceptibility of these phenotypes is different and that only a small number of loci are shared. Thus, we were able to capture genetic differences between LS and non-LS.

LS-associated variants expand through the extended MHC gene map (31), ranging from *ZNF184* to *HLA-DPB2*. Outside the extended MHC region, variants in *ADCY3*, *LRR16A*, and between *CSMD1* and *MCPH1* shall be further studied to explain their association, which may be linked to eQTL, as elucidated by *cis*-eQTL SNPs in gene expression of immunity cells (18, 21, 26, 32). The susceptibility of non-LS localized to a smaller region of the MHC class II, ranging from *C6orf10* to *HLA-DQA2*. Noteworthy, outside this region, confirmed variants highlighted exclusive associations with *PLAC9* and *ANXA11* (a previously discovered sarcoidosis-associated locus) on chromosome 10. An observed overlap between LS and non-LS defined by 17 variants that localized to MHC class II region is worth further exploration. Our efforts to gain insights on the role of *HLA-DRB1* alleles in non-LS and LS phenotypes reinforced these findings (results and further discussion are available in the NOTE in the online supplement). Likewise, results from a genome-wide gene-centric approach underlined the significance of LS and non-LS identified gene regions, which may well establish the groundwork for investigating these phenotypes independently.

Individual assessment of each gene variant based on bioinformatic exploration provides an extensive knowledge for supporting variant association by highlighting potential regulatory and epigenetic mechanisms ensuing within the associated regions.

As reported from many genome-wide association studies (33), common genetic variants can only explain a relatively small portion of the phenotype variability (explained variance in LS, 4.1–4.7%; non-LS, 1.28–2.13%) and heritability thus stressing the plausibility for involvement of other genetic and regulatory elements to account for the portion of phenotypic

variance. In non-LS, the results showed a presence of non-HLA genes, thus raising the possibility for *gene x factor* (a factor is defined as a gene, regulatory/epigenetic element, or protein) interactions across chromosomes, resembling a polygenic phenotype. Thus if so, the phenotype variability may be more likely explained by genes than rather by phenotype heterogeneity within non-LS (i.e., ocular, skin, heart, neurologic sarcoidosis, and many more), as we have demonstrated. Whereas in LS, the results show a higher presence of HLA-genes, suggesting that the phenotype variability may be explained by HLA-genes and *gene x factor* interactions located within one chromosome.

The presence of eQTL SNPs, regulatory elements, and epigenetic factors in the genomic regions associated with LS and non-LS, prompt new research questions that urge to be undertaken. eQTL genes have been proposed to act as master *cis*- and *trans*-regulators (34), whereas epigenetic elements alter chromatin structure and influence gene expression imposing specific heritable patterns on progeny cells (35–39). Based on this knowledge, it is not surprising to observe a predominant presence of *cis*-eQTL SNPs among the genetics of LS and non-LS. Analogously, the presence of epigenetic factors in the associated regions is expected given the numerous essential interactions between HLA and non-HLA genes. Hence, the observed implications and differences in

eQTLs and epigenetics events among LS and non-LS support our hypothesis that mechanisms underlying the genetic structure and pathogenesis of these phenotypes are distinct. The limitation of this bioinformatic analysis is the lack of expression and epigenetic data available from our datasets, which could facilitate an advanced understanding of biologic implications for the genomic-associated regions.

With the present collective information comprising associated variants and differently expressed genes in sarcoidosis, we are able to present a better understanding of the functional mechanisms that may shed light on the pathogenesis of LS and non-LS, and on the overlap between these phenotypes. The molecular similarities between both phenotypes may be elucidated by potential sharing mechanisms that were captured on pathway maps that identified several overlapping pathways underlining immune responses. For example, pathways maps highlighting immune response to naive CD4⁺ T, Th1, and Th2 cells shall be further studied to understand their correlation with these phenotypes. Particularly, in LS, pathway maps involving Th9, Th17, and Th22 cells and dendritic cells shall be favorably considered for investigation. Given that, significant involvement of Th17 cells determined by higher levels of IL-17 in bronchoalveolar lavage fluid of patients with LS has been reported (40). Naturally, to facilitate such investigations additional omics studies, such as transcriptomics

and epigenomics, are necessary before functional studies take place.

In summary, we present a comprehensive genetic study of LS and non-LS phenotypes of sarcoidosis in populations of European and African ancestry, thus focusing mainly on the genetic structure of each phenotype. For the first time, the genetic architecture of LS and non-LS is analyzed and presented, independently, identifying 175 loci associated with LS and 11 loci associated with non-LS by replication in independent cohorts. Moreover, out of the total number of loci identified, five are common to both phenotypes. We provide evidence to sustain our claim, that LS and non-LS are two different phenotypes with a shared genetic overlap and a reasonable number of specific factors. Through various analyses, we demonstrated that LS and non-LS have different genetic susceptibility and thus different genomic distributions that comprise distinctive gene regulatory elements associated with various relevant pathway maps of immune responses. ■

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