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Variants at multiple loci implicated in both innate and adaptive immune responses are associated with Sjögren's syndrome

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Abstract

Sjögren's syndrome is a common autoimmune disease (~0.7% of European Americans) typically presenting as keratoconjunctivitis sicca and xerostomia. In addition to strong association within the HLA region at 6p21 ($P_{meta}=7.65\times 10^{-114}$), we establish associations with *IRF5-TNPO3* ($P_{meta}=2.73\times 10^{-19}$), *STAT4* ($P_{meta}=6.80\times 10^{-15}$), *IL12A* ($P_{meta}=1.17\times 10^{-10}$), *FAM167A-BLK* ($P_{meta}=4.97\times 10^{-10}$), *DDX6-CXCR5* ($P_{meta}=1.10\times 10^{-8}$), and *TNIP1* ($P_{meta}=3.30\times 10^{-8}$). Suggestive associations with $P_{meta}<5\times 10^{-5}$ were observed with 29 regions including *TNFAIP3*, *PTTG1*, *PRDM1*, *DGKQ*, *FCGR2A*, *IRAK1BP1*, *ITSN2*, and *PHIP* amongst others. These results

highlight the importance of genes involved in both innate and adaptive immunity in Sjögren's syndrome.

Sjögren's syndrome is a common (~0.7% of European Americans¹), chronic, autoimmune condition characterized by exocrine gland dysfunction resulting in substantial morbidity. Females are affected at a rate of approximately 10 times more than males^{2,3}. Although patients are identified clinically by oral and ocular manifestations, the full disease spectrum may encompass a complex myriad of systemic features, making diagnosis a significant clinical challenge⁴. Current American-European Consensus Group classification criteria require either the presence of anti-Ro and/or -La autoantibodies or histologic evidence of inflammation within salivary glands plus other subjective and objective measures of keratoconjunctivitis sicca and xerostomia⁵. Therapeutic options are primarily palliative and do little to prevent irreversible exocrine gland damage⁶.

Sjögren's syndrome pathophysiology includes concurrent dysregulation of innate and adaptive immune pathways involving both cell-mediated and humoral disease processes that are incompletely understood⁷. Labial salivary gland and peripheral blood gene expression microarray studies have demonstrated dysregulation of type I interferon-inducible genes^{8,9}. Previous work in Sjögren's syndrome genetics is limited to candidate gene studies, yet strong association with several human leukocyte antigen (HLA) molecules, including *HLA-DR*, *HLA-DQB1*, and *HLA-DQAI*, has been established^{10,11}. Several non-HLA regions have been implicated in the disease, such as *IRF5* and *STAT4*¹²⁻¹⁵; however, none of these associations have exceeded the genome-wide significant (GWS) threshold of $P = 5 \times 10^{-8}$ (Supplementary Table 1). In this study, genome-wide association and large-scale replication approaches were used to identify new risk loci in a case-control cohort of primary Sjögren's syndrome patients.

Results

To maximize the power of our study, we formed the Sjögren's Genetics Network (SGENE), a collaborative research effort comprising multiple international sites (Supplementary Figure 1 and Supplementary Table 2). Dataset 1 (DS1) included 395 cases and 1975 population controls of European descent after implementing quality control (QC; see Online Methods and Supplementary Table 2). Genotyping was performed using the Illumina Omni1-Quad array. Dataset 2 (DS2) consisted of 1243 cases and 4779 population controls genotyped on the Illumina ImmunoChip, and Dataset 3 (DS3) included 1158 cases and 3071 population controls genotyped on a supplemental custom array designed to include variants not present on the ImmunoChip (all of European descent and after QC; Supplementary Figure 1b). Dataset 4 (DS4) evaluated 1541 cases and 2634 population controls genotyped on the Illumina ImmunoChip focusing on variants exclusive to DS4 and not common between DS1 and DS2 (Supplementary Figure 1b). Imputation was performed in regions with $P < 5 \times 10^{-5}$ to increase the number of overlapping variants available for analysis (Supplementary Figure 1c and 1d).

Human Leukocyte Antigen (HLA)

The HLA region at 6p21 was the only association that exceeded the GWS threshold of $P < 5 \times 10^{-8}$ in DS1 (Supplementary Figure 2). After imputation, we found the peak effect at rs112357081 near *HLA-DRA* with $P_{DS1} = 1.01 \times 10^{-32}$ (Table 1; Supplementary Figure 3). No inflation was observed in the test statistic ($\lambda_{GC} = 1.0$) and no significant deviation from the expected distribution of P -values was detected after removing the HLA region (Supplementary Figure 4). After meta-analysis between the imputed DS1 and DS2 (Figures 1 and 2; Supplementary Figure 1), strong association spanned a region that included the

HLA Class I, III, and II loci (Figure 2a; Supplementary Table 3), with peak association observed 5' of *HLA-DQB1* in the HLA Class II region at rs115575857 with $P_{meta}=7.65\times 10^{-114}$ (Table 1 and Figure 2a). A second set of variants not in linkage disequilibrium (LD) with rs115575857 was also identified peaking at rs116232857 ($P_{meta}=1.33\times 10^{-96}$; Table 1 and Figure 2a).

Further dissection through logistic regression analysis in DS2 adjusting for rs115575857 identified an ~5 Mb haplotype encompassing the extended HLA region (Supplementary Figure 5). In addition, a narrow region of residual association tagged by rs116232857 ($P_{residual}=7.00\times 10^{-26}$) was observed in the *HLA-DRA* through *HLA-DQA1* regions (Supplementary Figure 5). Logistic regression adjusting for rs115575857 and rs116232857 accounted for most of the association within the HLA region (the residual association in the region at rs115146037 went from 10^{-80} to 10^{-9} ; Supplementary Figure 5), thus identifying the presence of at least two independent effects.

Classical HLA alleles were imputed into our dataset to better understand the relationship between the HLA region variants tested in this study to those reported previously. The top associated classical allele was HLA-DQB1*0201 ($P_{meta}=1.38\times 10^{-95}$) followed closely by HLA-DQA1*0501 ($P_{meta}=8.50\times 10^{-94}$; Supplementary Table 4 and Supplementary Figure 6). We used logistic regression adjusting for both the top associated classical HLA alleles and the variants identified through the meta-analysis of DS1 and DS2. As previously described¹⁶, our data shows that HLA-DQB1*0201, HLA-DQA1*0501, and HLA-DRB1*0301 are in strong LD ($r^2>0.97$), and adjusted logistic regression using any of the three accounted for the observed association. Moreover, each of these classical alleles accounted for the association at rs115575857. Likewise, rs115575857 explained the association at each of the three classical alleles (Supplementary Figure 6). Adjusting for rs116232857 could account for the association at HLA-DQB1*0501, a previously reported protective allele in Sjögren's syndrome¹¹. However, HLA-DQB1*0501 could not account for the association observed at rs116232857, suggesting that rs116232857 better tags the functional effect(s) than the classical allele.

Enrichment analysis using all variants with $P_{meta}<5\times 10^{-5}$ identified a statistically significant association of disease-associated variants within 100 bp of regions found to be cross-linked to the transcription factor (TF) RFX5 ($P=1.53\times 10^{-14}$) by ENCODE ChIP-seq studies¹⁷. In total, 161 variants contributed to the enrichment in our dataset with all but one located in the HLA region. To further evaluate the influence of the extensive LD in the HLA, we tested for enrichment of Sjögren's syndrome-associated variants in RFX5 ChIP-seq peaks using only the HLA region variants present in DS1 as the background and continued to observe significant enrichment ($P=9.24\times 10^{-7}$; Supplementary Table 5).

To evaluate the functional mechanism of this RFX5 enrichment within the HLA the variants near RFX5 ChIP-seq peaks were tested as expression quantitative trait loci (eQTL), resulting in several statistically significant results (Figure 3a-e); Supplementary Table 6 and Supplementary Figure 7). The top overall eQTL was observed with rs114846898 and HLA-DRB6 ($P_{ANOVA}=1.03\times 10^{-42}$; Figure 3a). A modest eQTL was observed for rs116232857 and HLA-DRB6 ($P_{ANOVA}=1.77\times 10^{-12}$); however, we found that rs116232857 and rs114846898 were nearly in perfect LD with each other ($D'=0.98$), suggesting that the association at rs116232857 might be due to rs114846898. Looking at the adjusted analysis results, we found that rs115575857 and rs116232857 could explain rs114846898, but adjusting for rs115575857 and rs114846898 left residual association ($P_{residual}=9.44\times 10^{-7}$) at rs116232857, suggesting that rs116232857 likely tags additional functional variant(s).

In addition to the strong association observed in the HLA region, six non-HLA loci surpassed the GWS threshold, including *IRF5*, *STAT4*, *IL12A*, *BLK*, *CXCR5* and *TNIP1* (Table 2 and Figure 1).

Interferon Regulatory Factor 5 (IRF5)

The most statistically significant association outside the HLA region after meta-analysis was observed in the region of *IRF5*. In total, 67 SNPs exceeded the $P_{meta}=5\times 10^{-8}$ threshold, with the peak effect observed in the *IRF5* promoter region (rs3757387, $P_{meta}=2.73\times 10^{-19}$; Figure 2b; Supplementary Table 7 and Supplementary Figure 8). A group of variants, tagged by rs17339836 ($P_{meta}=2.43\times 10^{-16}$) and spanning *IRF5* and *TNPO3*, were not correlated with the promoter variants (Figure 2b).

Logistic regression adjusting for rs3757387 explained all of the promoter region association, while residual association persisted with variants spanning the *IRF5-TNPO3* genes tagged by rs17339836 ($P_{residual}=2.86\times 10^{-4}$; Supplementary Figure 9). Moreover, a group of variants tagged by rs6948875 that were not associated in the unadjusted single marker analysis became statistically significant when adjusting for rs3757387. Adjusting for both rs3757387 and rs17339836 again resulted in residual association of the region tagged by rs6948875 and rs10954213 (Supplementary Figure 9). Since rs10954213 was statistically significant in the single marker analysis (Supplementary Table 7), this SNP was added to the model in lieu of rs6948875. Logistic regression models adjusting for rs3757387, rs17339836, and rs10954213 resulted in no residual association in the regions, indicating the likelihood of three independent effects. Bioinformatics database mining of this region indicated that the associated variants were located within a variety of biologically relevant features encompassing a wide range of potential functional mechanisms (Supplementary Figure 10).

Signal Transducer and Activator of Transcription 4 (STAT4)

Statistically significant association exceeding GWS was observed between Sjögren's syndrome and 12 variants, 8 of which fall within the third intron of the *STAT4* locus, with peak association at an insertion-deletion (indel) polymorphism (rs10553577; $P_{meta}=6.80\times 10^{-15}$; Figure 2c; Supplementary Table 8 and Supplementary Figures 11 and 12). As expected, the indel rs10553577 accounted for the association within the region of *STAT4*; however, residual association was observed with rs3024918 ($P_{residual}=2.70\times 10^{-4}$) in the promoter region of *STAT1*, but did not surpass our required threshold ($P<1\times 10^{-4}$) after adjustment for an independent effect (Supplementary Figure 12). Bioinformatics databases revealed a large number of TFs bound to both regions (Supplementary Figure 13); however, neither effect resulted in a statistically significant eQTL (data not shown).

Interleukin 12A (IL12A)

Seven variants spanning the entire region from the promoter demonstrated strong association, with peak association occurring 5.3kb 3' of *IL12A* at rs485497 ($P_{meta}=1.17\times 10^{-10}$; Figure 2d; Supplementary Table 9 and Supplementary Figure 14). Logistic regression analysis adjusting for rs485497 indicated this variant accounted for all the observed association (Supplementary Figure 15). To determine the functional role of the variants associated between *IL12A* and Sjögren's syndrome, eQTL analysis was done for all variants showing evidence of association in the region. Of the variants exceeding GWS, only rs485497 was associated with *IL12A* transcript expression; however, seven variants 3' of *IL12A* influenced expression more significantly, with the eQTL peaking at rs4680536 (Figure 3f; Supplementary Table 6). Haplotype analysis of the *IL12A* associated variants indicates the presence of only one risk haplotype and the possibility that rs485497 may tag functional variants located on either side of this SNP (Supplementary Figure 15), which is

supported by the presence of multiple transcription factors and other regulatory elements located in the flanking LD blocks (Supplementary Figure 16).

B Lymphoid Kinase (BLK)

A total of 30 variants on chromosome 8 exceeded GWS in the region of *FAM167A* and *BLK* (Figure 2e; Supplementary Table 10 and Supplementary Figure 17). Maximum association after meta-analysis was observed in the shared promoter region of *FAM167A-BLK* at rs2736345 ($P_{meta}=4.97\times 10^{-10}$); however, this SNP was not statistically significant in DS1 (Table 2). The second most significant variant after meta-analysis (significant in both DS1 and DS2) was rs2729935 with $P_{meta}=6.85\times 10^{-10}$, which is located in the first intron of *BLK*. Logistic regression analysis adjusting for rs2729935 (as this variant was significant in both datasets) indicated residual association ($P_{residual}=1.75\times 10^{-3}$) present at rs2736345. However, adjusting for rs2736345 in the logistic model showed the likely presence of only one effect accounting for all the association in the region (Supplementary Figure 18).

Using expression data for *BLK*, eQTL analysis of the associated variants revealed several with the potential to influence transcript expression, including the top overall SNP in the region of *BLK*, rs2736345 (Supplementary Table 6). The most statistically significant eQTL was located in the first intron of *BLK* at rs2409781 (Figure 3g), a variant loosely correlated with rs2736345 and rs2729935 ($r^2\approx 0.5$). Evaluation of *FAM167A* probes with the variants associated with Sjögren's syndrome yielded greater statistical significance than those with *BLK* (Figure 3h). Bioinformatics analysis indicated the presence of many transcriptionally relevant markers not only in the shared promoter region, but also in the first intron of *BLK*, suggesting the possibility that association in this region impacts the transcriptional regulation of *BLK* and/or *FAM167A* (Supplementary Figure 19).

Chemokine (C-X-C motif) receptor 5 (CXCR5)

Association with a variant ~16 kb 5' of the coding region of *CXCR5* was observed at rs7119038 ($P_{meta}=1.10\times 10^{-8}$; Figure 2f; Supplementary Table 11 and Supplementary Figure 20). Three additional variants also exceeded the GWS threshold extending to rs11217033 located as far as ~90 kb 5' of *CXCR5* and as close to ~12 kb 5' of the neighboring gene, DEAD (Asp-Glu-Ala-Asp) box polypeptide 6 (*DDX6*) (Figure 2f). Adjusting for rs7119038 explained all the association signals in this region (Supplementary Figure 21). Although no statistically significant eQTLs were present in the region for *CXCR5*, bioinformatics analysis indicated the presence of many regulatory elements throughout the region (Supplementary Figure 22). Assessment of eQTLs for *DDX6* was not possible as the probe failed QC in the gene expression study.

TNFAIP3 interaction protein 1 (TNIP1)

In the region of *TNIP1*, one SNP, rs6579837, exceeded GWS with $P_{meta}=3.30\times 10^{-8}$; however, 29 additional variants showed evidence of association with $P_{meta}<5\times 10^{-5}$ (Figure 2g; Supplementary Table 12 and Supplementary Figure 23). Logistic regression analysis adjusting for rs6579837 accounted for all association observed in the region (Supplementary Figure 24). Several of the variants associated with Sjögren's syndrome were also eQTLs for the *TNIP1* probe, including rs6579837 (Supplementary Table 6). However, the most statistically significant eQTL was observed at rs73272842 located in *TNIP1*, where multiple regulatory elements have been identified (Figure 3i; Supplementary Figure 25).

Suggestive associations with Sjögren's syndrome

We identified 8 loci that were significant in meta-analysis ($P_{meta}<5\times 10^{-5}$) but did not surpass the overall GWS threshold (Supplementary Figure 1b and Table 3). Twenty-one

additional regions surpassed suggestive thresholds for significance ($P_{meta} < 5 \times 10^{-5}$) using DS4 (evaluated variants were exclusive to DS4; Supplementary Figure 1b; Supplementary Table 13). Further replication of these regions is needed to determine if they are bona fide risk loci.

Protein-protein interactions and pathway analysis

To assess the potential effects of associated variants on the underlying biology, we utilized two bioinformatics tools: the Disease Association Protein-Protein Link Evaluator (DAPPLE)¹⁸ and Genomatrix Pathway System (GePS)¹⁹. DAPPLE defines genomic regions in LD with variants achieving GWS and suggestive thresholds to determine any potential protein-protein interaction (PPI) that could elucidate the underlying biological perturbations in Sjögren's syndrome. In assessing the GWS variants outside the HLA region only, we observed no direct PPI, and while indirect PPI was observed, it was not more likely than expected by chance (Supplementary Figure 26). However, assessing both GWS and suggestive variants outside the HLA region, we observed a direct PPI network consisting of 9 proteins with 6 direct interactions ($P=0.0007$) and mean associated protein direct connectivity of 1.33 (expected=0.63, $P=0.048$) in addition to an indirect network that exhibited a mean associated protein indirect connectivity trending toward significance ($P=0.082$) (Supplementary Figures 27 and 28). Pathway analysis in GePS for the 6 GWS loci outside HLA found enrichment in immune signal transduction pathways (3/269 genes; $P=1.54 \times 10^{-3}$) and IL-12 signaling pathways (2/7 genes; $P=3.80 \times 10^{-5}$), primarily due to the influence of *IL12A* and *STAT4*. Adding the remaining 23 loci demonstrating suggestive association yielded similar results, with dominance of IL-12- and *STAT4*-dependent signaling ($P=1.63 \times 10^{-6}$). Assessing gene involvement in disease processes using Medical Subject Headings (MeSH) showed significant enrichment in multiple immunologically-mediated diseases, including non-Hodgkin lymphoma (16/2910 genes; $P=1.7 \times 10^{-6}$), systemic lupus erythematosus (SLE) (12/1691 genes; $P=4.74 \times 10^{-6}$), Epstein-Barr virus infections (11/1421 genes; $P=6.18 \times 10^{-6}$), dry eye syndrome (8/868 genes; $P=4.99 \times 10^{-5}$), and Sjögren's syndrome (6/756 genes; $P=1.15 \times 10^{-3}$).

Discussion

In this study, associations previously identified in the HLA region with Sjögren's syndrome were replicated and represent the strongest genetic risk factors with $OR \approx 3.5$. Our results are consistent with a trans-racial meta-analysis of 23 case-control studies evaluating Class II loci in Sjögren's syndrome, which also identified HLA-DQA1*0501, HLA-DQB1*0201, and HLA-DRB*0301 as disease risk factors¹¹. Associated variants in this study with a $P_{meta} < 5 \times 10^{-5}$ were found to be enriched within 100 bp of RFX5 ChIP-seq peaks identified by ENCODE¹⁷. Mutations in RFX5 can lead to the inability to transcribe Class II HLA molecules and bare lymphocyte syndrome²⁰. In Sjögren's syndrome, several risk alleles in or near the RFX5 ChIP-Seq peaks in the Class II region were eQTLs (Figure 3a-e), indicating that this TF may play a critical pathogenic role. Moreover, eQTLs were identified in the Class I region with additional HLA molecules, including *HLA-C*, *HLA-A*, *HLA-H*, and *HLA-G*, and previous studies have found that RFX5 regulates the expression of these loci²¹. Extensive LD between variants in the HLA region may influence the enrichment analysis performed in GenomeRunner when using all variants as background; however, we continued to observe enrichment when focusing only on the HLA region. Although we cannot completely rule out the influence of LD on these results, the observation that several of these variants are eQTLs further indicates biological relevance.

Although HLA Class II molecules have classically been implicated in Sjögren's syndrome risk, HLA Class I loci have also been reported²². This suggests that risk attributed to the

HLA region may involve altered expression of multiple genes residing in this region and important to the immune system through altered binding of RFX5 to DNA motifs and/or accessory molecules. However, it is likely that other mechanisms, such as amino-acid changes similar to those identified by Raychaudhuri et al. in rheumatoid arthritis (RA)²³, are involved in the genetic pathophysiology of this region. Comparing the effect sizes for the two primary HLA associations with other autoimmune diseases, we find that effect sizes in Sjögren's syndrome appear stronger than those in SLE and RA, similar to those in MS, and weaker than those in celiac disease, type I diabetes, and psoriasis (Supplementary Figure 29).

This study also established *IRF5-TNPO3*, *STAT4*, *IL12A*, *FAM167A-BLK*, *DDX6-CXCR5*, and *TNIP1* as risk loci. *IRF5* is a transcription factor mediating type I interferon responses in monocytes, dendritic cells, and B cells²⁴ that induces the transcription of interferon- α genes and the production of pro-inflammatory cytokines (including IL-12, p40, IL-6, and TNF- α)²⁵ upon viral infection. *IRF5* is an established risk locus in SLE, RA, ulcerative colitis, primary biliary cirrhosis (PBC), and systemic sclerosis (SSc)²⁶⁻³³. In SLE, association with three independent functional alleles was observed³⁴, similar to the effects described in this study. Previous studies in Sjögren's syndrome have also implicated *IRF5* as a risk locus, but only ~15 variants have been tested, with a CGGGG indel polymorphism yielding the most statistically significant result and appearing to be responsible for the promoter region association (Supplementary Table 1)^{13,15,35,36}. While we were unable to impute the CGGGG indel, the variant rs2004640, which is correlated ($r^2=0.7$) with the CGGGG indel³⁶, demonstrated association ($P_{meta}=8.86\times 10^{-15}$). Moreover, a three variant haplotype, consisting of rs3757385, rs2004640, and rs10954213, perfectly tags the CGGGG indel^{37,38}. Adjusting logistic regression models for these three variants showed significant residual association ($P=1.05\times 10^{-7}$) at rs3757387. After adding rs3757387, residual association ($P=1.64\times 10^{-4}$) was observed at rs17339836, suggesting that adjusting for the 3 SNP haplotype tagging the CGGGG indel was equivalent to adjusting for only rs10954213. Additional studies are necessary to determine the precise causal variant in the *IRF5* promoter region.

STAT4 is critical for cellular responses initiated by type I interferons and is subsequently induced by IL-12 in lymphocytes, leading to the transcription of interferon- γ ³⁹. The association of variants in the third intron of *STAT4* has been well established in other inflammatory diseases^{32,40-42} and has been implicated in Sjögren's syndrome (Supplementary Table 1)^{12,14,15,43}. The variants reported in these studies are highly correlated ($r^2>0.95$) with the associated variant rs10553577 identified in this study.

IL12A encodes the p35 subunit that forms the IL-12 heterodimer with the p40 subunit encoded by *IL12B*⁴⁴. IL-12, an immunomodulatory cytokine primarily secreted by monocytes and dendritic cells, plays a critical role in T-helper 1 cell differentiation and the production of interferon- γ by T cells and NK cells⁴⁵. Although no studies in Sjögren's syndrome report association to the *IL12A* region, several studies in related conditions have identified variants near *IL12A* that increase disease risk. Association of variants in the 3' end of *IL12A* have been reported for PBC, while 5' effects have been described for celiac disease⁴⁶⁻⁴⁸. Interestingly, the variants reported in PBC showed weak correlations ($r^2<0.35$) with the top SNP in Sjögren's syndrome, and analyses adjusting for variants identified in related diseases continued to exhibit residual association ($P_{residual}<0.05$) at rs485497. Moreover, rs574808, rs495499, and rs6441286, the most statistically significant variants in PBC^{42,46}, are highly correlated with the eQTL polymorphism reported in this study. Previous studies observed only suggestive association with the block encompassing the coding region of this gene, which includes the top overall associated variant, rs485497, in

this study. Thus, the effect in the region of *IL12A* in Sjögren's syndrome appears to be at least partially distinct from PBC.

IRF5, *IL12A*, and *STAT4*, all participate in type I interferon pathway signaling. A role for type I interferon pathway dysregulation in Sjögren's syndrome has been described through mRNA gene expression microarray studies of labial salivary glands and peripheral blood^{8,9,49,50}. Moreover, Emamian et al. has shown that overexpression of type I interferon inducible genes correlates with titers of the classic Sjögren's syndrome autoantibodies anti-Ro and anti-La⁹. Genetic effects in Sjögren's syndrome potentially influence both subunits of IL-12. *IRF5* can initiate transcription of *IL12B* while variants in the region of *IL12A* have also been identified as risk factors in this study. Once secreted from dendritic or NK cells, IL-12 binds T cell receptors, thereby initiating a signaling cascade through *STAT4* phosphorylation⁴⁴.

BLK is a non-receptor src family tyrosine kinase involved in B cell receptor signaling and B cell development. B cell receptor signaling is essential for proper immune function, deletion of autoreactive B cells, and subsequent receptor editing^{51,52}. Variants in the *BLK* locus have been reported to influence both *FAM167A* and *BLK* mRNA and protein expression and are associated with related diseases, such as SLE^{53,54}. Previous studies have implicated this region in Sjögren's syndrome risk with peak association observed within *FAM167A* at rs12549796¹⁴, while the current study identifies the shared promoter region and the first intron of *BLK* as the most statistically significant associations (Figure 2e).

CXCR5, a membrane-bound protein present on some memory B cells and on follicular helper T cells, acts as a receptor for B-lymphocyte chemoattractant (BLC). Variants in the region of *CXCR5* have been associated with multiple sclerosis and PBC^{42,55}. Although no genetic associations between Sjögren's syndrome and *CXCR5* have been reported, several studies have found *CXCR5* to be dysregulated in B cells in salivary gland tissues and the periphery^{49,56}. Interestingly, IL-12 alone can induce *CXCR5* expression, leading to the early commitment of naïve CD4⁺ T cells to the T follicular helper cell lineage⁵⁷.

Although the exact function of *TNIP1* has not been defined, *TNIP1* does bind *TNFAIP3*, which suppresses TLR-induced apoptosis by negatively regulating NF- κ B⁵⁸. Variants in the region of *TNIP1* have been found to increase risk for RA, SLE, psoriasis, and SSC⁵⁹⁻⁶³. In SLE, two independent risk haplotypes have been identified, with the H2 haplotype defined by Adrianto et al.⁶² corresponding to the association identified in this study. Similar to the findings reported above, the H2 haplotype in SLE also shows allele-specific differential expression⁶².

Considered jointly in the logistic regression model, the genome-wide and suggestively associated variants could explain a significant proportion of the heritable risk (*C* statistic = 0.811, Supplementary Table 14). This value is higher than that of the SLE model in women of European ancestry (*C* statistic = 0.67)³³. Consideration was also given to the effect size of Sjögren's syndrome-associated variants in LD with previously established loci achieving GWS in other autoimmune diseases. Of note, the OR and 95% CI for Sjögren's syndrome variants outside the HLA region (Supplementary Table 15 and Supplementary Figure 30) are comparable to those in related autoimmune diseases, particularly with respect to SLE, with much greater variability in effect size and CI when considering the variants in the HLA region (Supplementary Table 16 and Supplementary Figure 29).

Using bioinformatics tools, the combination of GWS and suggestively associated variants provides some evidence of significant direct and indirect PPI and enrichment of genes involved in immune signaling processes, particularly with respect to IL-12 and *STAT4* and

many autoimmune-related diseases. The importance of type I interferons has been previously recognized in the pathogenesis of Sjögren's syndrome. However, the type II interferon pathway, acting through interferon- γ downstream of IL-12 and STAT4, is now clearly implicated by this study. In addition, multiple genes in the NF- κ B pathway, including *TNIP1* and *TNFAIP3*, support a role for this pathway in Sjögren's syndrome pathogenesis. Furthermore, establishing association of several genes important in the adaptive immune response, including *CXCR5* and *BLK*, as well as suggestive associations in loci such as *FCGR2A*, provides insight into the complex genetic architecture for Sjögren's syndrome and will facilitate further studies to understand the precise functional consequences of these risk variants. Taken together, this work provides significant progress towards explaining the underlying genetic pathophysiology of Sjögren's syndrome, which will ultimately provide important new therapeutic targets and potential diagnostic markers, both of which are lacking.

In summary, we performed a large-scale genetic study in Sjögren's syndrome and established *IRF5-TNPO3*, *STAT4*, *IL12A*, *FAM167A-BLK*, *DDX6-CXCR5*, and *TNIP1* as risk loci. We have determined that there are likely multiple independent effects within the HLA and *IRF5* regions. Our results also strongly implicate 29 suggestive regions in Sjögren's syndrome etiology warranting further study. Together, these results highlight the importance of the innate and adaptive immune systems in Sjögren's syndrome etiology. Future work is needed to replicate these additional candidate associations and to characterize the causal variant(s) for the regions established in this study.

Online Methods

Subjects

The samples used in this study were collected through the Sjögren's Genetics Network (SGENE) and organized at the Oklahoma Medical Research Foundation (OMRF; Supplementary Table 2). In Dataset 1, 438 Sjögren's syndrome cases and 3917 population controls of European descent were subjected to quality control measures outlined below. Each Sjögren's syndrome case was genetically matched to five population controls in DS1 using the identity-by-state (IBS) to assess allele sharing as implemented in PLINK⁶⁴, and the remaining controls were used in DS2 and DS3. In the next phase, 1521 Sjögren's syndrome cases and 6626 population controls were evaluated in DS2 only for variants that overlapped with DS1; 1169 Sjögren's syndrome cases and 3078 population controls were evaluated in DS3; and 1897 cases and 3039 population controls were evaluated in DS4 (313 cases overlapped with DS1). Cases fulfilled the American-European Consensus Group (AECG) criteria for primary Sjögren's syndrome⁵, and all population controls were evaluated within their respective study. All cases were evaluated by expert clinicians for possible overlap of clinical features or concurrent diagnosis with other autoimmune diseases. Written informed consent was obtained by each participant following protocols approved by the Institutional Review Boards of each institution where the samples were collected.

Genotyping and Quality Control

Genotypes for DS1 were obtained using the Illumina Omni1-Quad array using Infinium chemistry at OMRF following the manufacturer's protocol (Illumina, Inc., San Diego, CA). Similarly, DS2/DS4 genotyping was performed with the ImmunoChip (an Illumina iSelect custom array designed by the ImmunoChip Consortium)⁶⁵. DS3 was genotyped on iSelect arrays containing 1536 variants overlapped with DS1 that were not available on the ImmunoChip. Strict quality control procedures were implemented before SNPs were used in this analysis, including requirements for: well-defined cluster scatter plots; minor allele

frequency >1%; SNP call rate >95%; sample call rate >95%; Hardy-Weinberg proportion (HWP) test with a $P > 0.001$ in controls; and $P > 0.001$ for differential missingness between cases and controls.

Samples with <95% call rate and excessive increased heterozygosity (>5 standard deviations from the mean) were excluded from the analysis. Relatedness within the remaining samples was determined using identity-by-descent (IBD) estimates as determined by PLINK v1.07⁶⁴. One individual from each pair was removed if the proportion of the alleles shared identity by descent was >0.4, with preference towards cases and/or the individual with the highest call rate. Gender was estimated genetically, and discrepancies with self-report were scrutinized. Males were required to be heterozygous at rs2557523 (since the G allele for this SNP is only observed on the Y chromosome and the A allele appears only on the X chromosome) and to have chromosome X heterozygosity = 10%, while females were required to be homozygous for the A allele at rs2557523 and have chromosome X heterozygosity >10%. After quality control, 648,937 SNPs were available to test for association across all datasets for DS1. Only SNPs present on both DS1 and DS2 arrays (20,055 after quality control) were used for meta-analysis and fine-mapping (after imputation, see below for more details). All base pair positions are given according to the hg19 version of the human genome reference sequence.

Ascertainment of Population Stratification

Genetic outliers were removed from further analysis as determined by principal components analysis (Supplementary Figure 31)^{66,67}. Population substructure was identified within the sample set using EIGENSTRAT⁶⁶ with 40,073 (DS1) and 2,827 (DS2/DS4) independent markers ($r^2 < 0.20$ between variants). The resulting Eigenvectors were able to distinguish the four continental ancestral populations in the following HapMap samples: Africans (ASW, LWK, MKK, and YRI), Europeans (CEU and TSI), Hispanic and East Indians (MEX and GIH), and Asians (CHB, CHD, and JPT); Supplementary Figure 30)^{67,68}. We utilized principal components to identify samples output by EIGENSTRAT that were not of European ancestry. Samples >6 standard deviations from the mean were eliminated from further analysis. Samples were then plotted by PC1 and PC2 for only the cases and controls used in DS1, DS2, or DS4 (Supplementary Figure 31). We further scrutinized and removed outliers that resided outside the main cluster of cases and controls. In addition, each Sjögren's syndrome case was genetically matched to 5 out-of-study population controls for DS1 using the cluster feature in PLINK v1.07⁶⁴. After quality control, 395 Sjögren's syndrome cases and 1975 population controls in DS1, 1243 cases and 4779 controls in DS2, 1158 cases and 3071 controls on DS3, and 1541 case and 2634 controls in DS4 were available to test for association (Supplementary Figure 1 and Supplementary Table 2).

Statistical Analysis

To test for SNP-Sjögren's syndrome association, logistic regression models were computed as implemented in PLINK v1.07⁶⁴. The additive genetic model was calculated for chromosomes 1-22 while adjusting for the first three principal components (as these accounted for >80% of the variation in the dataset after quality control) and gender. For chromosome X, only females were tested for association with Sjögren's syndrome. Meta-analysis of the SNPs observed in both DS1 and DS2 or DS3 were calculated using a weighted Z-score in METAL⁶⁹. Each dataset group was weighted by the square root of its sample size to control for differences between the two phases. For a locus to be considered it must have been significant in both datasets and the P_{meta} must have exceeded the suggestive threshold of $P_{meta} < 5 \times 10^{-5}$.

To test for meta-analysis heterogeneity, we utilized both the Cochran's Q test statistic⁷⁰ and I^2 index⁷¹ (reported in the Supplementary Tables 3, 7-12). A $P < 0.05$ was considered

significant evidence of heterogeneity for Cochran's Q test. The I^2 index ranges between 0% and 100%, where I^2 equal 0% to 25%, 26% to 50%, 51% to 75%, and 76% to 100%, indicating low, moderate, high, and very high heterogeneity, respectively. These data are provided in the Supplementary Tables for each region (Supplementary Tables 3, 7-12).

Linkage disequilibrium and probable haplotypes were determined using HAPLOVIEW ver. 4.2⁷². Haplotype blocks were calculated using the solid-spine of LD algorithms with minimum r^2 values of 0.8⁷². To determine the number of independent effects in each region, we used step-wise logistic regression adjusting for the most significant variant with $P < 0.0001$ in the both the adjusted and unadjusted analysis to be considered. Adjusted logistic regression analysis results for each Sjögren's syndrome-associated region were plotted using LocusZoom⁷³.

We computed the C statistic of the multiple logistic regression model using "rms" package in R. The C statistic is the area under the receiver operator characteristic (ROC) curve that provides a measure on how well the model can discriminate between cases and population controls.

Imputation

To increase informativeness, imputation was conducted in subjects from both the DS1 and DS2 over a 100 kb interval spanning loci that met criteria for genome-wide significance or select loci demonstrating suggestive significance. Imputation of both datasets was performed across 12 regions: *FCGR2A* (chr1: 161,425,205-161,539,360), *STAT4* (chr2: 191,844,302-192,066,322), *IL12A* (chr3: 159,356,623-159,763,806), *DGKQ* (chr4: 923,459-1,001,272), *TNIP1* (chr5: 150,359,504-150,517,221), *PTTG1* (chr5: 159,528,253-159,902,455), *HLA* (chr6: 28,400,000-33,400,000), *TNFAIP3* (chr6: 137,938,325-138,254,451), *IRF5* (chr7: 128,527,994-128,740,088), *BLK* (chr8: 11,251,521-11,472,108), *PRDM1* (chr6: 106,484,195-106,607,814), and *DDX6-CXCR5* (chr11: 118,548,473-118,816,980). Imputation was performed using IMPUTE2 and the European Impute2 1000 Genomes Phase 1 April 2012 reference panel⁷⁴⁻⁷⁶. Imputed genotypes had to meet or exceed a probability threshold of 0.9, an information measure of >0.5 , and the quality control criteria described above to be included in the analyses.

The HLA classical allele imputation was performed using HLA Genotype Imputation with Attribute Bagging (HIBAG)⁷⁷. HIBAG is a highly accurate, computationally tractable package for imputing HLA types using SNP data without a training dataset in various populations, including European population. By using HLA SNPs after QC, we imputed the HLA classical alleles in seven HLA genes in both DS1 and DS2 (Supplementary Figure 6). Individuals with imputation score < 0.8 and alleles with frequency < 0.01 were removed for analysis.

Expression Quantitative Trait Loci (eQTL) Analysis

Gene expression profile analysis: We performed global gene expression profiling (GEP) analysis using peripheral blood on 48,803 probes representing 37,805 loci from the Illumina HumanWG-6 v3.0 BeadChip in 258 subjects: 182 primary Sjögren's syndrome cases meeting AECG 2002 criteria⁵ and 76 population controls. Analyses were performed in the R Bioconductor suite. The GEP data were obtained from two separate microarray experiments. Quality control (QC) was applied in each dataset to filter out transcripts that were expressed in less than 10% of the subjects (detection call threshold: $P < 0.05$) and probes with differential missing rates ($P < 0.001$ by Fisher's exact test) between the two datasets. Re-annotation and Mapping of Oligonucleotide Array Technologies (ReMOAT) was used to determine the quality of Illumina BeadArray probes⁷⁸. Each dataset was then normalized

independently using Robust Multiarray Average (RMA)⁷⁹ followed by \log_2 transformation and quantile normalization. ComBat was subsequently applied to the combined dataset to adjust for non-biological experimental variation (batch effect)⁸⁰. After QC and normalization, 15,063 probes (in 12,248 genes) were kept for further statistical analysis.

Cis-expression quantitative trait loci analysis: Expression levels of the Sjögren's syndrome-associated genes identified in the meta-analysis of DS1 and DS2 exceeding GWS were selected for use as phenotypic traits in *cis*-eQTL analyses in 222 European subjects (190 cases and 32 controls). Variants associated with Sjögren's syndrome ($P < 5 \times 10^{-5}$) in the meta-analysis flanking the target genes were evaluated for genetic association with gene expression levels using both a linear model in Matrix-eQTL⁸¹ (using gender and disease status as covariates) and Analysis of Variance (ANOVA) models in Prism 6. An eQTL was considered significant if a false discovery rate (FDR) adjusted *P*-value, accounting for the number of tests performed in each region, was $P < 0.05$.

Enrichment analysis

GenomeRunner⁸² was used to search for statistically significant enrichment of the set of 9,394 SNPs showing suggestive ($P < 5 \times 10^{-5}$) or lower association with Sjögren's syndrome with annotated genomic features. Genome annotations include genes and gene prediction tracks, mRNA and EST tracks, regulation related tracks from ENCODE project, histone modification marks, comparative genomics tracks, structural variation and repeats tracks. GenomeRunner evaluates the null hypothesis that co-localization of a set of Sjögren's syndrome-associated SNPs with genome annotation features is not statistically significantly different from what would be observed for a random set of SNPs of the same size. Briefly, the Sjögren's syndrome-associated set of SNPs was tested for co-localization with each of the genome annotation tracks obtained from the UCSC genome database⁸³ for the human GRCh37/hg19 genome assembly. The whole set of 219,958 SNPs after imputation of select regions present on the ImmunoChip array was used to randomly select an equally sized group.

Separate analyses included identification of overrepresented experimentally validated TFBSs transcription factor binding sites, a total of 148 of them extracted from the "Transcription Factor ChIP-seq from ENCODE" track (wgEncodeRegTfbsClustered). Regions of Chromatin State Segmentation by HMM from ENCODE/Broad (wgEncodeBroadHmmGm12878HMM) were also used to assess overrepresentation of defined chromatin states containing Sjögren's syndrome-associated SNPs. Tracks of Histone Modifications obtained by ChIP-seq from ENCODE/Broad Institute, from ENCODE/Stanford/Yale/USC/Harvard, and from ENCODE/University of Washington were used for assessment of epigenetic marks. Unless otherwise noted, data from the GM12878 lymphoblastoid cell line were used, as the majority of the best quality genome annotation data was obtained from this cell line. The number of Sjögren's syndrome-associated SNPs co-localized with a genome annotation track was compared with that of obtained from 1,000 random simulations using Chi-square test. Additional analyses of random sets of SNPs showed no statistically significant enrichments (data not shown). For an enrichment to be considered statistically significant, we set a Bonferroni correction threshold for 6000 independent tests at $P < 8.33 \times 10^{-6}$.

RFX5-HLA region enrichment analysis

The HLA region was defined by genomic coordinates chr6:28400295-33398620 (GRCh37/hg19 human genome assembly). The number of significant SNPs in this region overlapping with RFX5 binding sites was calculated using BedTools⁸⁴. The number of all genome-wide associated SNPs in the HLA region overlapping RFX5 binding sites was calculated and

compared with the SNPs significantly associated with Sjögren's syndrome using Fisher's exact test (`scipy.stats.fisher_exact` function).

Protein-protein interactions

The Disease Association Protein-Protein Link Evaluator (DAPPLE)¹⁸ was used to assess the potential effects of associated variants on the underlying biology. For DAPPLE, seed variants were converted to genes based on variant overlap and LD structure within the region of interest. Analyses were performed using the following settings: 10,000 permutations; a common interactor binding degree cutoff of 2; gene regulatory region 50kb upstream and downstream of transcription start and end; simplification of the indirect network; and iteration to prioritize genes with a Bonferroni-corrected score of $P < 0.05$. A total of 14 GWS variants and 114 suggestive variants were present in the DAPPLE databases; where variants were not found to be present, attempts to find proxies in high LD were made.

Pathway Analysis in Genomatix

Pathway analysis was carried out using the Genomatix Pathway System (GePS)¹⁹. This system utilizes information obtained from public and proprietary annotation databases to characterize input gene lists, determine the representation of these genes in canonical pathways, and create gene networks based on co-citation of genes from the literature. The public annotation data sets incorporated in this software include gene ontology (GO) and medical subject headings (MeSH) in addition to others. GePS also provides a probability statistic using Fisher's exact test to determine the likelihood of finding the number of co-represented genes with a particular annotation when considering the length of the input gene list. Input gene sets were generated using the gene locus nearest the association signals, which were required to demonstrate suggestive or genome-wide significance, and the pathway analysis was carried out using the gene symbols of the input set.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Footnotes

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Data access.

URLs.

HLA Genotype Imputation with Attribute Bagging <http://cran.r-project.org/web/packages/HIBAG/>

R Bioconductor suite <http://www.R-project.org>

Prism 6 <http://www.graphpad.com/scientific-software/prism/>

GenomeRunner <http://sourceforge.net/projects/genomerunner>

Genomatix Pathway System <http://www.genomatix.de>

Accession Numbers: Genes and Blood Clotting Study dataset(s) obtained through dbGaP: phs000304.v1.p1
 GWAS of Ischemic Stroke dataset(s) obtained through dbGaP: phs000292.v1.p1
 CIDR:NGRC Parkinson's disease study dataset(s) obtained through dbGaP: phs000196.v2.p1
 High Density SNP Association Analysis of Melanoma: Case-Control and Outcomes Investigation dataset(s) obtained through dbGaP: phs000187.v1.p1

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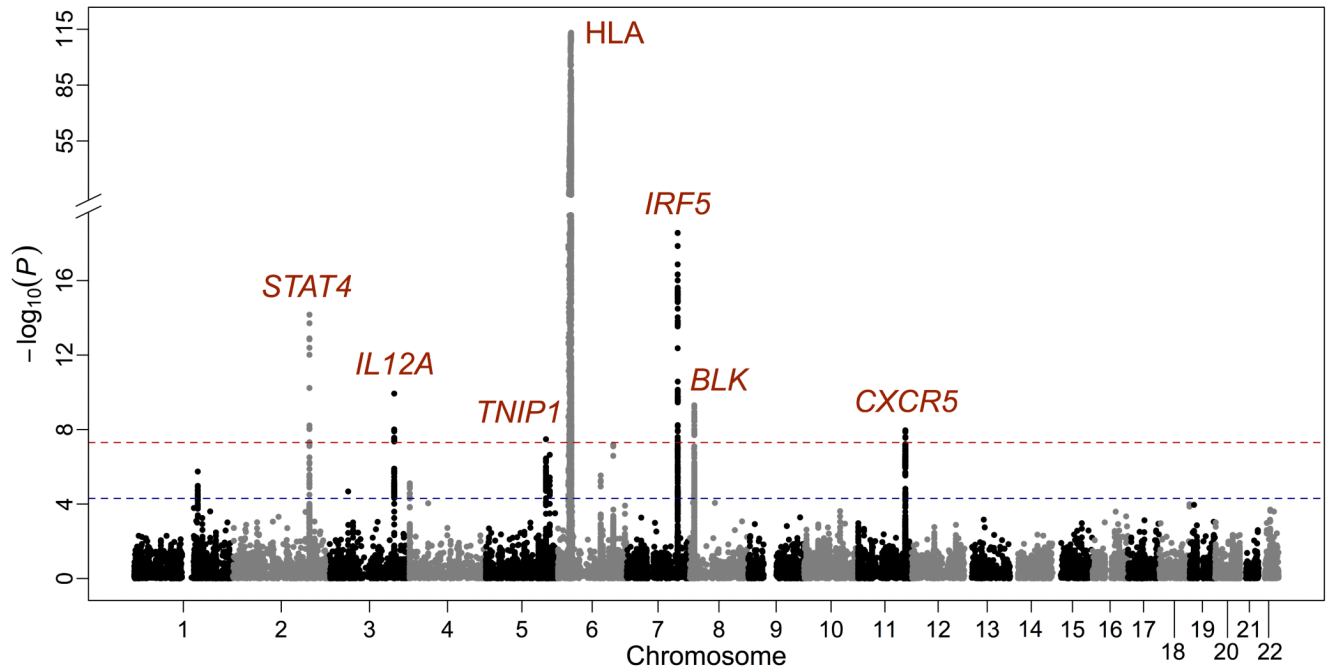


Figure 1.

Summary of genome-wide association results for 27,501 variants overlapping between DS1 and DS2 after imputation and meta-analysis. The $-\log_{10}(P)$ for each variant is plotted according to chromosome and base pair position. A total of seven loci (in red font) exceeded the GWS of $P_{meta} < 5 \times 10^{-8}$ (red dashed line). Suggestive threshold ($P_{meta} < 5 \times 10^{-5}$) is indicated by a blue dashed line.

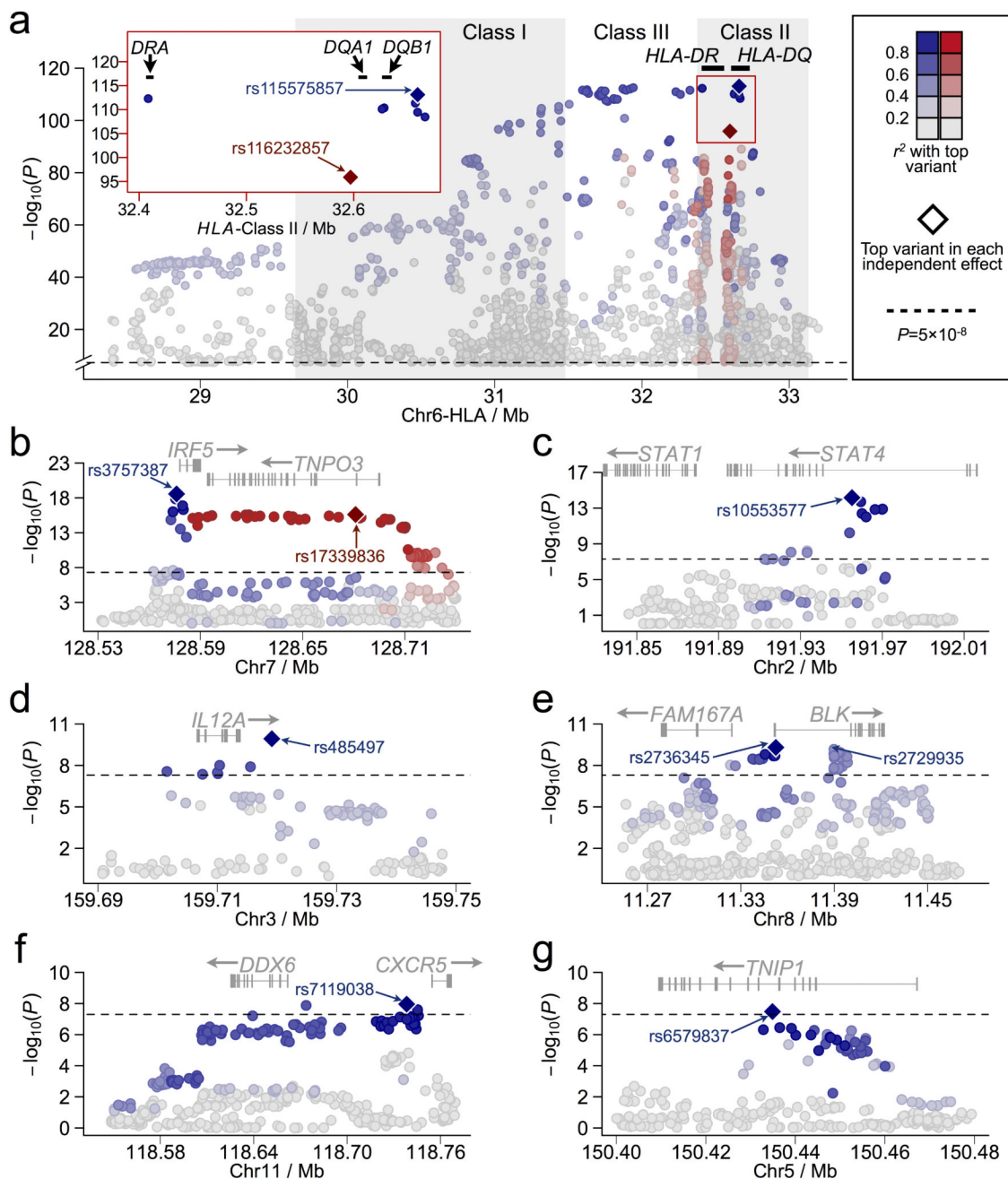


Figure 2. Zoomed in plots of the meta-analysis results for the seven regions with $P_{meta} < 5 \times 10^{-8}$. The HLA region (a) from Mb 28.4 to Mb 33.4 is shown for variants with $P_{meta} < 5 \times 10^{-8}$ only. Strong linkage disequilibrium (LD) was observed with variants extending from Class I through Class II (pairwise LD with rs115575857 is given in blue). A second independent effect was identified in Class II peaking at rs116232857 (pairwise LD is depicted in red). The red insert on the left shows a further zoomed in view of the top associated variants in the Class II region. (b) The area of association surrounding *IRF5* is shown, including the pairwise LD with the top overall variant, rs3757387, in blue. Pairwise LD with the top SNP,

rs17339836, in the second effect is shown in red. *STAT4* (c), *IL12A* (d), *BLK* (e), *CXCR5* (f) and *TNIP1* (g) regions are also provided and include pairwise LD with the top listed variant shown in blue. The black dashed lines represent $P_{meta} = 5 \times 10^{-8}$.

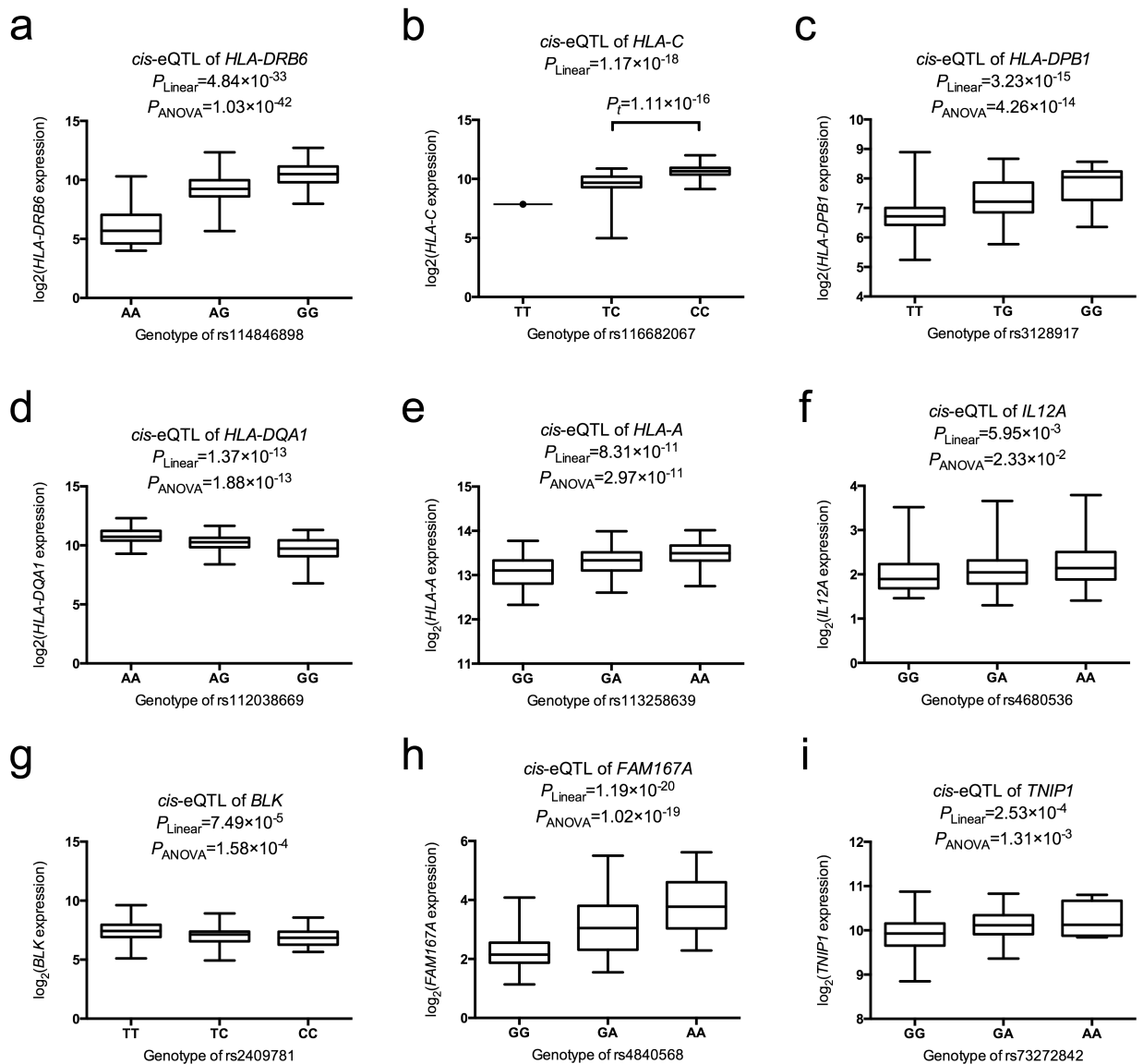


Figure 3. Identification of *cis*-eQTL in Sjögren's syndrome-associated regions. Sjögren's syndrome-associated variants in the HLA region within 100bp of RFX5 binding sites were evaluated for *cis*-eQTL with the expression of HLA genes in 222 European subjects. The top *cis*-eQTL in *HLA-DRB6* (a), *HLA-C* (b), *HLA-DPB1* (c), *HLA-DQA1* (d), and *HLA-A* (e) are shown in the figure. We also identified *cis*-eQTL for top Sjögren's syndrome-associated variants in *IL12A* (f), *FAM167A-BLK* (g, h), and *TNIP1* (i). The risk genotype of each eQTL variant is plotted on the right side of each figure. Data are shown using box-and-whisker plots, in which distribution of the expression values are highlighted (median, upper quantile, lower quantile, maximum, and minimum). Each eQTL was evaluated using both a linear model and Analysis of Variance (ANOVA) model. Panel b only had one subject with the TT genotype; thus, we performed a t-test rather than an ANOVA to compare the TC to the CC genotype.

Table 1

Top associations in the HLA region

SNP	Alleles		MAF ^b	Observed or Imputed in Dataset 1/2	Dataset 1		Dataset 2		Meta analysis	
	maj/min ^a	Case			Control	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
<i>HLA-DRA</i>										
rs112357081	TCTAA/T	0.59	0.33	I/-	2.89 (2.43-3.44)	1.01×10 ⁻³²	-	-	-	-
rs3135394	A/G	0.27	0.11	I/O	3.52 (2.83-4.38)	2.47 ×10 ⁻²⁹	3.52 (3.10-3.99)	1.14×10 ⁻⁸⁵	3.52 (3.02-4.10)	5.22×10 ⁻¹¹³
<i>HLA-DQB1</i>										
rs115575857	A/G	0.29	0.12	I/I	3.25 (2.61-4.05)	4.10 ×10 ⁻²⁶	3.65 (3.22-4.14)	3.70×10 ⁻⁹⁰	3.53 (3.03-4.11)	7.65×10 ⁻¹¹⁴
rs3129716	T/C	0.29	0.12	I/O	3.29 (2.66-4.09)	2.11 ×10 ⁻²⁷	3.51 (3.10-3.97)	7.74×10 ⁻⁸⁷	3.45 (2.97-4.00)	4.59×10 ⁻¹¹²
<i>HLA-DQA1</i>										
rs116232857	A/G	0.64	0.42	I/I	2.83 (2.37-3.38)	9.05 ×10 ⁻³¹	2.42 (2.19-2.67)	1.14×10 ⁻⁶⁷	2.53 (2.24-2.86)	1.33×10 ⁻⁹⁶
rs9271588	T/C	0.27	0.48	I/O	0.35 (0.29-0.42)	1.95 ×10 ⁻²⁸	0.43 (0.39-0.48)	4.62×10 ⁻⁵⁹	0.41 (0.36-0.46)	1.37×10 ⁻⁸⁵

^aMajor allele / minor allele. Odds ratio for each variant indicates disease risk conferred by the minor allele.^bThe minor allele frequency (MAF) was calculated using the combined Datasets 1 and 2, except for rs112357081, for which the MAF is derived from Dataset 1 only.

Table 2
Non-HLA regions associated with Sjögren's syndrome exceeding genome-wide significant threshold

SNP	Alleles		MAF ^b		Observed or Imputed in Dataset 1/2	Dataset 1			Dataset 2			Meta analysis		
	maj/min ^a		Case	Control		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P	
<i>IRF5</i> (Chr 7)														
rs3757387	T/C		0.54	0.45	I/I	1.31 (1.11-1.54)	1.49 × 10 ⁻³	1.50 (1.37-1.64)	7.50 × 10 ⁻¹⁸	1.44 (1.29-1.62)	2.73 × 10 ⁻¹⁹			
rs4728142	G/A		0.53	0.44	O/O	1.29 (1.09-1.52)	2.85 × 10 ⁻³	1.46 (1.33-1.60)	2.05 × 10 ⁻¹⁵	1.40 (1.25-1.57)	9.75 × 10 ⁻¹⁷			
rs17339836	C/T		0.18	0.12	O/O	1.65 (1.32-2.06)	8.53 × 10 ⁻⁶	1.55 (1.37-1.75)	5.73 × 10 ⁻¹²	1.58 (1.36-1.84)	2.43 × 10 ⁻¹⁶			
rs17338998	C/T		0.18	0.12	I/I	1.64 (1.32-2.05)	9.51 × 10 ⁻⁶	1.55 (1.37-1.75)	5.70 × 10 ⁻¹²	1.57 (1.35-1.83)	2.67 × 10 ⁻¹⁶			
rs10954213	A/G		0.34	0.38	O/O	0.83 (0.70-0.98)	2.91 × 10 ⁻²	0.82 (0.74-0.90)	3.63 × 10 ⁻⁵	0.82 (0.73-0.92)	3.20 × 10 ⁻⁶			
<i>STAT4</i> (Chr 2)														
rs10553577	TATA/T		0.30	0.23	I/I	1.38 (1.15-1.65)	5.13 × 10 ⁻⁴	1.45 (1.31-1.61)	2.30 × 10 ⁻¹²	1.43 (1.26-1.62)	6.80 × 10 ⁻¹⁵			
rs13426947	G/A		0.24	0.19	O/O	1.34 (1.11-1.62)	2.44 × 10 ⁻³	1.31 (1.18-1.47)	1.09 × 10 ⁻⁶	1.32 (1.16-1.51)	9.45 × 10 ⁻⁹			
<i>IL12A</i> (Chr 3)														
rs485497	G/A		0.54	0.48	O/O	1.32 (1.12-1.55)	9.47 × 10 ⁻⁴	1.30 (1.18-1.42)	3.15 × 10 ⁻⁸	1.30 (1.16-1.46)	1.17 × 10 ⁻¹⁰			
rs583911	A/G		0.48	0.42	I/I	1.29 (1.10-1.52)	2.16 × 10 ⁻³	1.26 (1.15-1.38)	1.28 × 10 ⁻⁶	1.27 (1.13-1.42)	9.88 × 10 ⁻⁹			
<i>BLK</i> (Chr 8)														
rs2736345	A/G		0.36	0.29	I/I	1.16 (0.97-1.37)	1.01 × 10 ⁻¹	1.37 (1.24-1.50)	2.76 × 10 ⁻¹⁰	1.30 (1.16-1.47)	4.97 × 10 ⁻¹⁰			
rs2729935	C/A		0.41	0.35	I/I	1.28 (1.08-1.52)	4.02 × 10 ⁻³	1.30 (1.19-1.43)	4.29 × 10 ⁻⁸	1.30 (1.16-1.46)	6.85 × 10 ⁻¹⁰			
rs6998387	G/A		0.37	0.32	O/I	1.34 (1.13-1.59)	6.75 × 10 ⁻⁴	1.23 (1.12-1.36)	2.65 × 10 ⁻⁵	1.26 (1.12-1.42)	7.96 × 10 ⁻⁸			
<i>CXCR5</i> (Chr 11)														
rs7119038	A/G		0.18	0.23	I/I	0.79 (0.64-0.98)	3.32 × 10 ⁻²	0.72 (0.64-0.81)	6.33 × 10 ⁻⁸	0.74 (0.64-0.86)	1.10 × 10 ⁻⁸			
rs4936443	T/C		0.16	0.20	O/O	0.79 (0.63-0.98)	3.21 × 10 ⁻²	0.74 (0.65-0.83)	5.03 × 10 ⁻⁷	0.75 (0.65-0.87)	6.82 × 10 ⁻⁸			
<i>TNIP1</i> (Chr 5)														
rs6579837	G/T		0.12	0.09	I/I	1.58 (1.23-2.04)	3.94 × 10 ⁻⁴	1.38 (1.19-1.59)	1.71 × 10 ⁻⁵	1.43 (1.20-1.71)	3.30 × 10 ⁻⁸			
rs7732451	A/G		0.15	0.12	O/O	1.36 (1.09-1.71)	6.77 × 10 ⁻³	1.33 (1.17-1.52)	2.43 × 10 ⁻⁵	1.34 (1.14-1.57)	5.32 × 10 ⁻⁷			

^aMajor allele / minor allele. Odds ratio for each variant indicates disease risk conferred by the minor allele.

^bThe minor allele frequency (MAF) was calculated using the combined Datasets 1 and 2.

Table 3

Suggestive regions associated with Sjögren's syndrome

Gene symbol	Chr	SNP	Alleles		MAF ^b	Observed or Imputed in Dataset 1 / 2 or 3 ^c	Dataset 1		Dataset 2 or 3		Meta analysis	
			maj/min ^a	Control			Case	OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)
<i>Replicated using Dataset 2</i>												
<i>TNFAIP3</i>	6	rs6933404	T/C	0.26	0.21	O/O	1.18 (0.97-1.43)	9.27×10 ⁻²	1.34 (1.20-1.49)	1.02×10 ⁻⁷	1.29 (1.13-1.47)	6.53×10 ⁻⁸
<i>TNFAIP3</i>	6	rs35926684	G/A/G	0.27	0.22	I/I	1.23 (1.01-1.49)	3.65×10 ⁻²	1.31 (1.18-1.46)	4.47×10 ⁻⁷	1.29 (1.13-1.47)	7.21×10 ⁻⁸
<i>PTTG1</i>	5	rs2431098	G/A	0.45	0.51	I/I	0.83 (0.71-0.97)	1.90×10 ⁻²	0.80 (0.73-0.88)	3.54×10 ⁻⁶	0.81 (0.73-0.91)	2.28×10 ⁻⁷
<i>PTTG1</i>	5	rs2431697	T/C	0.39	0.43	O/O	0.85 (0.72-1.00)	4.75×10 ⁻²	0.82 (0.75-0.90)	2.49×10 ⁻⁵	0.83 (0.74-0.93)	3.76×10 ⁻⁶
<i>PRDMI</i>	6	rs26531	G/A	0.36	0.31	O/O	1.25 (1.05-1.49)	1.10×10 ⁻²	1.21 (1.10-1.34)	8.71×10 ⁻⁵	1.22 (1.09-1.38)	2.93×10 ⁻⁶
<i>PRDMI</i>	6	rs498679	C/T	0.35	0.31	I/I	1.26 (1.06-1.50)	8.29×10 ⁻³	1.20 (1.09-1.32)	2.07×10 ⁻⁴	1.22 (1.08-1.37)	5.46×10 ⁻⁶
<i>DGKQ</i>	4	rs3733346	T/C	0.54	0.49	I/I	1.18 (1.01-1.39)	4.00×10 ⁻²	1.20 (1.32-1.10)	6.56×10 ⁻⁵	1.20 (1.22-1.18)	7.73×10 ⁻⁶
<i>DGKQ</i>	4	rs4690326	C/A	0.42	0.47	O/O	0.85 (0.72-1.00)	4.47×10 ⁻²	0.83 (0.76-0.91)	8.08×10 ⁻⁵	0.84 (0.75-0.93)	1.05×10 ⁻⁵
<i>FCGR2A</i>	1	rs6658353	C/G	0.45	0.49	I/I	0.77 (0.66-0.91)	1.96×10 ⁻³	0.86 (0.78-0.94)	1.13×10 ⁻³	0.83 (0.75-0.93)	1.06×10 ⁻⁵
<i>FCGR2A</i>	1	rs10800309	G/A	0.30	0.34	O/O	0.85 (0.71-1.01)	6.94×10 ⁻²	0.84 (0.76-0.92)	3.62×10 ⁻⁴	0.84 (0.75-0.95)	6.72×10 ⁻⁵
<i>Replicated using Dataset 3</i>												
<i>IRAK1BPI</i>	6	rs1507153	C/A	0.41	0.36	O/O	1.33 (1.13-1.57)	5.93×10 ⁻⁴	1.22 (1.09-1.35)	2.91×10 ⁻⁴	1.26 (1.11-1.43)	7.09×10 ⁻⁷
<i>ITSN2</i>	2	rs1545257	C/T	0.44	0.48	O/O	0.74 (0.63-0.87)	2.42×10 ⁻⁴	0.85 (0.77-0.94)	1.71×10 ⁻³	0.81 (0.71-0.91)	2.47×10 ⁻⁶
<i>PHIP</i>	6	rs10943608	T/C	0.41	0.37	O/O	1.30 (1.11-1.53)	1.49×10 ⁻³	1.19 (1.07-1.32)	1.09×10 ⁻³	1.23 (1.08-1.40)	6.22×10 ⁻⁶

^aMajor allele / minor allele. Odds ratio for each variant indicates disease risk conferred by the minor allele.

^bThe minor allele frequency (MAF) was calculated using the combined datasets.

^cObserved (O) or Imputed (I) variant in Dataset 1 / Dataset 2 or 3 dataset. Both the top observed and imputed variants for each locus are given for those replicated using Dataset 2.