Polygenic analysis of schizophrenia and 19 immune diseases reveals modest pleiotropy

Running Title: Pleiotropy between schizophrenia and autoimmunity

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Abstract

Epidemiological studies have revealed that schizophrenia and autoimmune diseases co-occur in the general population at higher than expected rates. Here, we evaluated whether the epidemiologic correlation between immune diseases and schizophrenia might be explained by shared genetic risk factors. We first evaluated the association of 581 variants previously reported to be associated with 19 different immune diseases at genome-wide significance in a recent genome-wide association study (GWAS) of schizophrenia (N=35,476 cases and 46,839 controls). We identified nine variants with pleiotropic effects, associated with both schizophrenia and autoimmunity. Five of these pleiotropic variants were located outside of the HLA region, and mapped to genes with known roles in calcium signaling. We then evaluated whether polygenic risk scores for immune diseases, which take into account the collective effects of all SNPs ($p<1$ in association with the immune disease of interest), predicted schizophrenia. Among 14 immune diseases with available genome-wide summary statistics, higher polygenic risk scores for narcolepsy (liability-scale R^2=0.02%, $p=4.1x10^{-4}$), primary biliary cirrhosis (R^2=0.04%, $p=1.4x10^{-8}$), psoriasis (R^2=0.02%, $p=3.6x10^{-5}$), systemic lupus erythematosus (R^2=0.04%, $p=2.2x10^{-8}$), type 1 diabetes (R^2=0.03%, $p=2.0x10^{-6}$), and ulcerative colitis (R^2=0.02%, $p=4.3x10^{-4}$) were significantly associated with schizophrenia. We also observed suggestive evidence of sex-dependent pleiotropy between schizophrenia and multiple sclerosis (interaction p=0.02), with genetic risk scores for multiple sclerosis associated with greater risk of schizophrenia among males but not females. Our findings reveal the presence of modest pleiotropy between schizophrenia and several autoimmune diseases, which in some cases may be sex-dependent.

Introduction

Despite recent advances in identifying key biomarkers and genetic loci for schizophrenia, its pathophysiology remains poorly understood.[1, 2] One interesting epidemiological observation is that the risk of developing an autoimmune disease is increased among patients with schizophrenia,[3-5] and vice versa.[6, 7] While there are discrepancies among studies regarding which autoimmune diseases are most strongly correlated with schizophrenia, there is converging evidence that these diseases co-occur at a greater rate than is expected by chance.[3-7] A notable exception is rheumatoid arthritis (RA), where a consistent inverse association with schizophrenia has been observed.[5, 8]

The epidemiological co-occurrence of immune diseases and schizophrenia suggests there may be shared disease processes, potentially resulting from shared genetic risk factors. Clarifying whether this is the case could shed new light on the pathophysiology of schizophrenia, and may be of considerable clinical benefit. Recently, we found that susceptibility to schizophrenia does not appear to be driven by the broad set of loci harboring immune genes.[9] However, not all genetic variants conferring risk of autoimmune disease fall within immune loci, and the extent of genetic overlap between schizophrenia and autoimmune diseases remains to be fully explored. Here, we evaluated whether shared genetic risk factors contribute to the co-occurrence of immune diseases and schizophrenia.

Table 1. Description of datasets analyzed

	Abr	Genome-wide significant SNPs[a]	Polygenic risk scoring[b]	Cases	Controls	Total number of SNPs		
						Full GWAS	*Merged with SCZ*[c]	*Pruned*[d]
Schizophrenia	SCZ	-	Target[1]	35,476	46,839	-	-	-
Height	HGT	-	Negative control[26]	253,288	-	2,085,602	2,035,446	124,888
Alopecia areata	AA	11	-	-	-	-	-	-
Ankylosing spondylitis	AS	23	-	-	-	-	-	-
Autoimmune thyroid disease	ATD	7	-	-	-	-	-	-
Celiac disease	CEL	38	Training[10]	12,041	12,228	133,352	90,922	19,698
Crohn's disease	CRO	119	Training[11]	5,956	14,927	12,276,506	4,990,991	114,950
Inflammatory bowel disease	IBD	145	Training[11]	12,882	21,770	12,716,150	5,095,448	116,346
Juvenile idiopathic arthritis	JIA	22	Training[12]	772[e]	8,530[e]	122,330	98,477	20,337
Multiple sclerosis	MS	103	Training[13]	14,498	24,091	155,756	108,118	21,818
Narcolepsy	NAR	3	Training[14]	1,886	10,421	109,768	92,859	19,866
Primary biliary cirrhosis	PBC	19	Training[18]	2,764	10,475	1,038,537	1,041,977	97,806
Primary sclerosing cholangitis	PSC	12	-	-	-	-	-	-
Psoriasis	PSO	34	Training[19]	2,178	5,175	7,586,779	3,701,354	107,002
Rheumatoid arthritis	RA	77	Training[15]	5,539	20,169	2,090,825	2,087,383	126,049
Sjögren's syndrome	SJO	6	-	-	-	-	-	-
Systemic lupus erythematosus	SLE	19	Training[16]	4,036	6,959	7,915,251	6,539,217	264,374
Systemic sclerosis	SSC	4	Training[21]	1,486[f]	3,477[f]	253,179	251,441	66,402
Type 1 diabetes	T1D	56	Training[17]	9,340[g]	12,835	123,081	98,418	20,835
Ulcerative colitis	UC	96	Training[11]	6,968	20,464	12,255,263	5,167,266	120,720
Vitiligo	VIT	16	Training[22]	1,381	14,518	8,790,155	6,223,502	257,654

[a]We obtained lists of genome-wide significant SNPs for each autoimmune disease from ImmunoBase, and processed them as described in **Supplementary Methods**; [b]The following columns provide details for datasets used in the polygenic risk scoring analysis. We used effect sizes obtained from the height (negative control) and autoimmune disease GWASs (training datasets) to construct polygenic risk scores in the schizophrenia sample (target dataset). Because genome-wide summary statistics were required for this analysis, we were unable to perform polygenic risk scoring for five autoimmune diseases for which these data were not available (AA, AS, ATD, PSC, SJO); [c]Prior to merging the training dataset SNP set with the target schizophrenia dataset SNP set, the following quality control steps were performed: SNPs on non-

autosomal chromosomes (X, Y, M) were removed, SNPs with MAF<0.01 were removed if MAF was available in the training dataset, SNPs with INFO<0.90 were removed if INFO was available in the training dataset, SNPs with missing p-value or OR were removed, symmetrical SNPs were removed; [d]Pruning was performed by clumping using PLINK to retain SNPs with r^2<0.1 within 1,000 kb windows, while filtering for the highest significance levels within LD blocks (using options --clump-p1 1 --clump-p2 1 --clump-r2 0.1 --clump-kb 1000); [e]only the UK cohort from this study was available for analysis; [f]only the US cohort from this study was available for analysis; [g]includes cases from 2,601 affected sibling pairs and 69 trios, which were analyzed using the Generalized Disequilibrium Test (GDT) method and combined with case-control results by meta-analysis; Abr, abbreviation; -, not analyzed.

Materials and Methods

Samples and quality control

We used either imputed genotype data or summary statistics generated as described in the original genome-wide association studies (GWASs). For sample details, see **Table 1**.

Schizophrenia dataset

We used data from the SCZ-52 GWAS.[1] For analyses of genome-wide significant risk variants for autoimmune diseases we used publicly available summary statistics from the total dataset (52 cohorts; 35,476 cases and 46,839 controls).[1] For polygenic risk scoring (PRS) analyses we used all 36 European ancestry case-control cohorts with available individual-level genotype data (25,629 cases and 30,976 controls). For analyses including HLA variants we used a further refined 31 European ancestry case-control cohorts (20,253 cases and 25,011 controls) with high-quality coverage of the MHC region, as previously described.[9]

Immune disease datasets

To estimate the extent of genetic overlap between schizophrenia and immune diseases, we obtained full GWAS or Immunochip summary statistics for 14 of the 19 immune diseases (five immune diseases were not included in PRS analyses due to lack of available summary statistics). We obtained publicly available summary statistics for ten immune diseases (see URLs): celiac disease (CEL),[10] Crohn's disease (CRO),[11] inflammatory bowel disease (IBD),[11] juvenile idiopathic arthritis (JIA),[12] multiple sclerosis (MS),[13] narcolepsy (NAR),[14] RA,[15] systemic lupus erythematosus (SLE),[16] type 1 diabetes (T1D),[17] and ulcerative colitis (UC).[11] For an additional four immune diseases, we obtained summary statistics with permission from the authors: primary biliary cirrhosis (PBC),[18] psoriasis (PSO),[19,20] systemic sclerosis (SSC),[21] and vitiligo (VIT).[22]

Testing the association of genome-wide significant risk alleles for 19 immune diseases in schizophrenia

For each of the 19 immune diseases, we defined risk loci outside of the major histocompatibility complex (MHC) region (chromosome 6: 25-34 Mb) using curated GWAS results from ImmunoBase (for details, see **Supplementary Methods**). Notably, we included the union of risk loci for CRO and UC as IBD loci. Within the MHC region we considered only the most strongly associated human leukocyte antigen (HLA) variant (including SNPs, imputed HLA amino acid sites, and classical alleles) for each disease based on univariate analysis in previously published studies (see **Table 2**), because conditional analyses reporting adjusted effect sizes of independent HLA variants were not available for all immune diseases. In total there were 581 unique variants (563 non-HLA variants and 18 HLA variants) associated with any autoimmune disease at genome-wide significance.

Table 2. Association of top HLA variants for immune diseases in schizophrenia

		Autoimmune		Schizophrenia		
Disease	HLA variant	*p*	OR	*p*	OR	r^2 with top SCZ SNP[a]
Alopecia areata[51]	**HLA-DRB1#37Asn**	**4.99×10^{-73}**	**0.42**	**4.85×10^{-9}**	**0.91**	**0.04**
Ankylosing spondylitis[52]	HLA-B*27	$<1\times10^{-100}$	46	0.13	1.05	0
Autoimmune thyroid disease[53]	rs2281388 (tags HLA-DPB1*05:01)	1.50×10^{-65}	1.64	0.39	1.04[b]	0
Celiac disease[54]	**HLA-DQB1#74Ala**	**n.r.**	**2.14**	**2.16×10^{-12}**	**0.89**	**0.11**
Crohn's disease[55]	HLA-DRB1*01:03	9.12×10^{-62}	2.51	0.61	0.96	0
Inflammatory bowel disease[55]	HLA-DRB1*01:03	1.93×10^{-112}	3.01	0.61	0.96	0
Juvenile idiopathic arthritis[12]	rs7775055	3.14×10^{-174}	6.01	0.12	0.94	0
Multiple sclerosis[56]	HLA-DRB1*15:01	1.40×10^{-234}	2.92	5.10×10^{-3}	1.06	0
Narcolepsy[57]	HLA-DQB1*06:02	1.04×10^{-120}	251	7.30×10^{-3}	1.06	0
Primary biliary cirrhosis[58]	HLA-DQA1*04:01	5.90×10^{-45}	3.06	0.20	0.95	0
Primary sclerosing cholangitis[59]	**HLA-B*08:01**	**3.70×10^{-246}**	**2.82**	**5.65×10^{-16}**	**0.84**	**0.2**
Psoriasis[60]	HLA-C*06:02	2.10×10^{-201}	3.26	0.55	0.99	0
Rheumatoid arthritis[61]	HLA-DRB1#11Val	$<1\times10^{-581}$	3.80	2.68×10^{-4}	1.07	0
Sjögren's syndrome[62]	**HLA-DQB1*02:01**	**1.38×10^{-95}**	**3.36**	**3.84×10^{-15}**	**0.85**	**0.11**
Systemic lupus erythematosus[63]	HLA-DRB1#13Arg	7.99×10^{-10}	1.55[c]	5.81×10^{-4}	1.07	0
Systemic sclerosis[64]	rs17500468 (TAP2)	5.87×10^{-62}	2.87	6.76×10^{-4}	1.07	0
Type 1 diabetes[65]	HLA-DQB1#57Ala	$<1\times10^{-1000}$	5.17	7.80×10^{-4}	0.95	0.06
Ulcerative colitis[55]	rs6927022	8.00×10^{-154}	1.49	3.37×10^{-4}	1.06	0.03
Vitiligo[22]	rs12206499 (tags HLA-A*02)	1.24×10^{-19}	1.58	6.97×10^{-3}	1.04[d]	0.06

[a]r^2 with rs12333578, the top HLA variant in schizophrenia, was obtained from the GAIN schizophrenia cohort (mgs2); [b]Effect size estimate is for HLA-DPB1*05:01; [c]Effect size estimate obtained from Asian sample; [d]Effect size estimate is for HLA-A*02.

We evaluated the association of these 581 variants with schizophrenia using previously published association results for non-HLA[1] and HLA variants.[9] We considered SNPs associated with schizophrenia at $p<8.6x10^{-5}$ (Bonferroni correction for 581 tests, 563 non-HLA and 18 HLA variants) to have pleiotropic effects.

We tested for shared direction of effect with schizophrenia among SNPs associated with each of the 19 immune diseases using the binomial sign test. Because some immune risk SNPs were associated with multiple diseases with inconsistent direction of effect, we could not evaluate shared direction of effect among the collective set of immune risk SNPs in schizophrenia.

To evaluate the collective association of SNPs associated with any immune disease, we evaluated the p-values of a pruned set of 429 LD-independent, non-HLA immune risk SNPs in the schizophrenia dataset. We quantified enrichment of immune risk SNP associations in schizophrenia using the genomic inflation value λ. We obtained an empirical enrichment p-value by comparing this to λ values from 1,000 equal-sized sets of SNPs drawn from the schizophrenia GWAS summary data, and matched to the immune SNP set for minor allele frequency (MAF) and linkage disequilibrium (LD) score as these parameters are correlated with GWAS test statistics (see **Supplementary Methods** for details).

Testing the association of polygenic risk scores for 14 immune diseases in schizophrenia

To evaluate whether common variants influencing risk of immune diseases collectively contribute to schizophrenia, we used PRS.[23, 24] To benchmark the amount of genetic overlap between schizophrenia and immune disease, we included previously published results for bipolar disorder as a positive control.[25] We used human height[26] as a negative control because – despite the inverse epidemiological relationship between height and schizophrenia previously reported[27, 28] – a recent study using cross-trait LD Score regression (LDSC) reported no genetic correlation with schizophrenia.[29]

For 14 immune diseases with available genome-wide summary statistics we performed PRS at a range of p-value thresholds (p_T)[25]: $5x10^{-8}$, $1x10^{-4}$, $1x10^{-3}$, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, and 1.0 (which included all LD-independent SNPs, **Table 1**). Due to extensive LD in the HLA region, we performed analyses both including the top HLA variant and excluding the HLA region. At each p_T, we constructed PRSs for each individual *i* in the schizophrenia cohort for each immune disease *h* by calculating the sum of risk-allele dosages (g) weighted by their effect sizes (β) for that immune disease:

$$PRS_{i,h} = \sum_{M} \beta_{M,h} g_{M,i}$$

where *M* iterates over all known risk alleles for disease *h*, $\beta_{M,h}$ is the effect size (log odds ratio) of *M* in disease *h*, and $g_{M,i}$ is the risk-allele dosage of *M* in individual *i*. We then performed logistic regression in R[30] using the stats package[30] to evaluate the association between schizophrenia case-status and PRSs for each immune disease. As in previous studies, statistical significance of the PRSs was estimated based on their logistic regression coefficient.[23, 25] Variance in schizophrenia case-status explained by the PRSs was estimated using the deviation in liability-scale R^2 between a null model (including 10 ancestry-informative principal components and study site) and the full model (including PRSs in addition to these covariates), calculated as previously described[31] assuming a population prevalence of schizophrenia of 1%. We also estimated Nagelkerke's pseudo-R^2 using the fmsb package.[32] We considered immune diseases with PRS $p<1.8x10^{-3}$ at any p_T

to show significant genetic overlap with schizophrenia (Bonferroni correction for 14 immune diseases tested in both sexes, $0.05/(14*2)=1.8x10^{-3}$). As in previous studies[23, 25] we did not use Bonferroni correction for the number of p-value thresholds, as these tests are highly correlated.

We excluded eight schizophrenia cohorts using Wellcome Trust Case Control Consortium (WTCCC) controls, due to the use of these samples in the immune disease GWASs. The total schizophrenia sample analyzed by PRS included 37,655 subjects (28 cohorts; 17,000 cases and 20,655 controls). Sex-stratified and formal sex-PRS interaction analyses were performed among the subset of subjects with known sex (9,787 male cases and 9,284 male controls; 5,231 female cases and 9,094 female controls). For details of PRS, see **Supplementary Methods** and **Table 1**.

Statistical power

Power to detect association of individual non-HLA and HLA immune risk variants in schizophrenia was calculated using the Genetic Power Calculator[33] assuming a risk allele frequency (RAF) of 0.05, disease prevalence of 1%, and significance threshold (α) of $8.6x10^{-5}$. Power for PRS was evaluated using AVENGEME,[34, 35] assuming disease and genetic parameters detailed in **Supplementary Table 1**.

Results

Genome-wide significant immune disease loci are associated with schizophrenia

Outside of the MHC region the number of genome-wide significant risk loci for each of the 19 immune diseases varied from three (NAR) to 144 (IBD), with a total of 563 unique risk variants associated with any immune disease (several variants were associated with more than one immune disease). Given the size of the schizophrenia GWAS, we had over 80% power to detect pleiotropic SNPs assuming an OR≥1.12 in schizophrenia. Five variants showed pleiotropic effects, with the risk allele for immune disease also conferring risk for schizophrenia (**Table 3**). These pleiotropic variants have been previously implicated in CRO (rs6738825 *PLCL1*, rs13126505 *BANK1*, rs1734907 *EPO*),[36, 37] MS (rs7132277 *PITPNM2*),[13] and CEL (rs296547 *C10orf106*).[38] Overall, the direction of effect for the 19 sets of SNPs associated with each immune disease at genome-wide significance was not shared with schizophrenia more than expected by chance (all binomial sign test p>0.05, **Supplementary Figure 1**).

Next, we evaluated whether there was overall enrichment of the non-HLA immune disease risk variants in schizophrenia. We evaluated the association of 429 LD-independent, non-HLA “immune risk SNPs,” defined as those associated with at least one of the 19 immune-mediated diseases. We found significant deviation from the theoretical null in schizophrenia for immune risk SNPs (λ=1.53).

Table 3. Immune disease risk SNPs showing pleiotropic effect in schizophrenia

SNP (chr:bp)	Immune Disease	Risk Allele/ Non-Risk Allele	Immune OR (95% CI); p	Schizophrenia OR (95% CI); p	Closest Gene	eQTL[a]	Function
rs6738825 (chr2: 198896895)	CRO[36]	A/G	1.06 (1.02-1.11); $3.50x10^{-9}$	1.05 (1.03-1.07); $3.02x10^{-6}$	*PLCL1* (intronic)	*PLCL1*, decreased expression *RFTN2*, decreased expression	Regulates $GABA_A$ receptor signaling[66], inhibits IP_3 mediated calcium signalling[67]
rs13126505 (chr4: 102865304)	CRO[b,37]	A/G	1.17 (1.10-1.25); $2.33x10^{-10}$	1.14 (1.10-1.19); $1.19x10^{-8}$	*BANK1* (intronic)	*BANK1*, decreased expression[c]	B-cell-specific scaffold protein that mediates receptor-induced calcium mobilization from intracellular stores[68]
rs1734907 (chr3: 100315517)	CRO[b,37]	A/G	1.16 (1.11-1.21); $1.67x10^{-13}$	1.07 (1.04-1.10); $7.55x10^{-6}$	*EPO* (2.9kb 5')	*EPHB4*, decreased expression **_GIGYF1_, increased expression** Other genes	Found in plasma, regulates red cell production by promoting erythroid differentiation and initiating hemoglobin synthesis; neuroprotective activity[69]; increases intracellular calcium concentration[70]
rs7132277 (chr12: 123593382)	MS[13]	A/G	1.12 (n.r.); $1.90x10^{-13}$	1.07 (1.04-1.09); $2.52x10^{-6}$	*PITPNM2* (intronic)	*ABCB9*, increased expression Other genes	Replenishes PIP_2 in plasma membrane, the major substrate for IP_3-related calcium channel activation[71]
rs296547 (chr1: 200892137)	CEL[38]	G/A	1.12 (1.09-1.16); $4.11x10^{-9}$	1.04 (1.02-1.07); $6.17x10^{-5}$	*C10orf106* (7.3kb 3')	*C10orf106*, inconsistent direction of effect across tissues Other genes	Unknown function

[a]cis-eQTL data from The GTEx Consortium[72] (http://gtexportal.org) and Westra *et al.*[73] (http://www.genenetwork.nl/bloodeqtlbrowser/); all genes with FDR<0.05 are listed, effect on expression (increased/decreased) corresponds to the risk allele; [b]Also associated with inflammatory bowel disease; [c]eQTL results presented are for proxy SNP rs13127398, r^2=0.89 with rs13126505; Disease abbreviations as defined in **Table 1**; bold fond indicates brain eQTLs.

However, when we compared the association of immune risk SNPs to that of similar SNP sets (**Supplementary Methods**) we observed no evidence of enrichment (**Supplementary Figure 2**, p=0.66), indicating that immune risk SNPs were not associated with schizophrenia more than expected by chance given the polygenic nature of the disease.

We also evaluated the association of HLA variants implicated in autoimmune disease. Overall, the effect sizes of these HLA variants were substantially greater for immune diseases (OR=1.19-251; median=2.87) than schizophrenia (OR=1.01-1.19; median=1.06). We observed four HLA risk alleles associated with both immune disease and schizophrenia, particularly in the class II HLA region (**Table 2, Figure 1**). The four HLA variants showing potential pleiotropic effects in schizophrenia were the strongest HLA risk variants for alopecia (HLA-DRB1 #37 Asn), CEL (HLA-DQB1 #74 Ala), primary sclerosing cholangitis (HLA-B*08:01), and Sjögren's syndrome (HLA-DQB1*02:01). There was very low LD between these four HLA variants and rs1233578, the strongest associated variant in the region in schizophrenia (r^2=0.04 – 0.20, **Table 2**). The presence of HLA-DRB1 #37 Asn conferred a protective association in both alopecia and schizophrenia, but the remaining HLA variants showed the opposite direction of effect in schizophrenia compared to immune disease (**Table 2**, **Figure 1**).

Polygenic risk for six immune diseases is associated with schizophrenia

For the PRS analyses we had over 80% power to detect genetic covariance with schizophrenia ranging from 0.02-0.03 for most of the immune diseases, although some showed less than 80% power in this range (PSO, SLE, VIT; **Supplementary Figure 3**).

Genetic scores including the HLA region were significant for CEL, NAR, PBC, PSO, RA, SLE, SSC, T1D, and UC ($p<1.8x10^{-3}$ at multiple p_T, **Supplementary Table 2**). With the exception of CEL ($\beta_{GRS}\approx$-0.04 at $p_T<5x10^{-8}$, $1x10^{-4}$, and $1x10^{-3}$), all immune diseases exhibited a positive association with schizophrenia case-status (all $\beta_{GRS}>0$, **Supplementary Table 2**). For CEL, RA, SLE, and SSC only those PRSs constructed using the most stringent p-value cutoffs ($5x10^{-8}$, $1x10^{-4}$, $1x10^{-3}$) were significantly associated with schizophrenia. To evaluate whether the HLA region alone was driving the observed genetic sharing, we constructed PRSs excluding this region. After excluding HLA variants, genetic scores for NAR, PBC, PSO, SLE, T1D, and UC remained significantly associated with schizophrenia (**Figure 2**, **Supplementary Table 3**). Given that the genetic overlap between these diseases and schizophrenia was not driven by a single HLA variant of large effect, we focused on these findings for the remainder of our analyses.

To benchmark the genetic sharing with schizophrenia observed for these six immune diseases, we compared the variance in schizophrenia case-status explained by their PRSs to that of bipolar disorder (positive control) and height (negative control). As expected, the immune disease PRSs explained substantially less variance in schizophrenia case-status (all liability-scale R^2<0.06%, **Figure 2**, **Supplementary Table 3**) than that previously observed for bipolar disorder PRSs (liability-scale R^2=0.88% at $p_T<1$, **Figure 2**).[25] Surprisingly, immune diseases also explained less variance in schizophrenia case-status than that observed for human height (liability-scale R^2=0.06% at $p_T<1$, **Figure 2**). Height was analyzed as a negative control based on its previously reported lack of genetic correlation with schizophrenia using LDSC.[29]

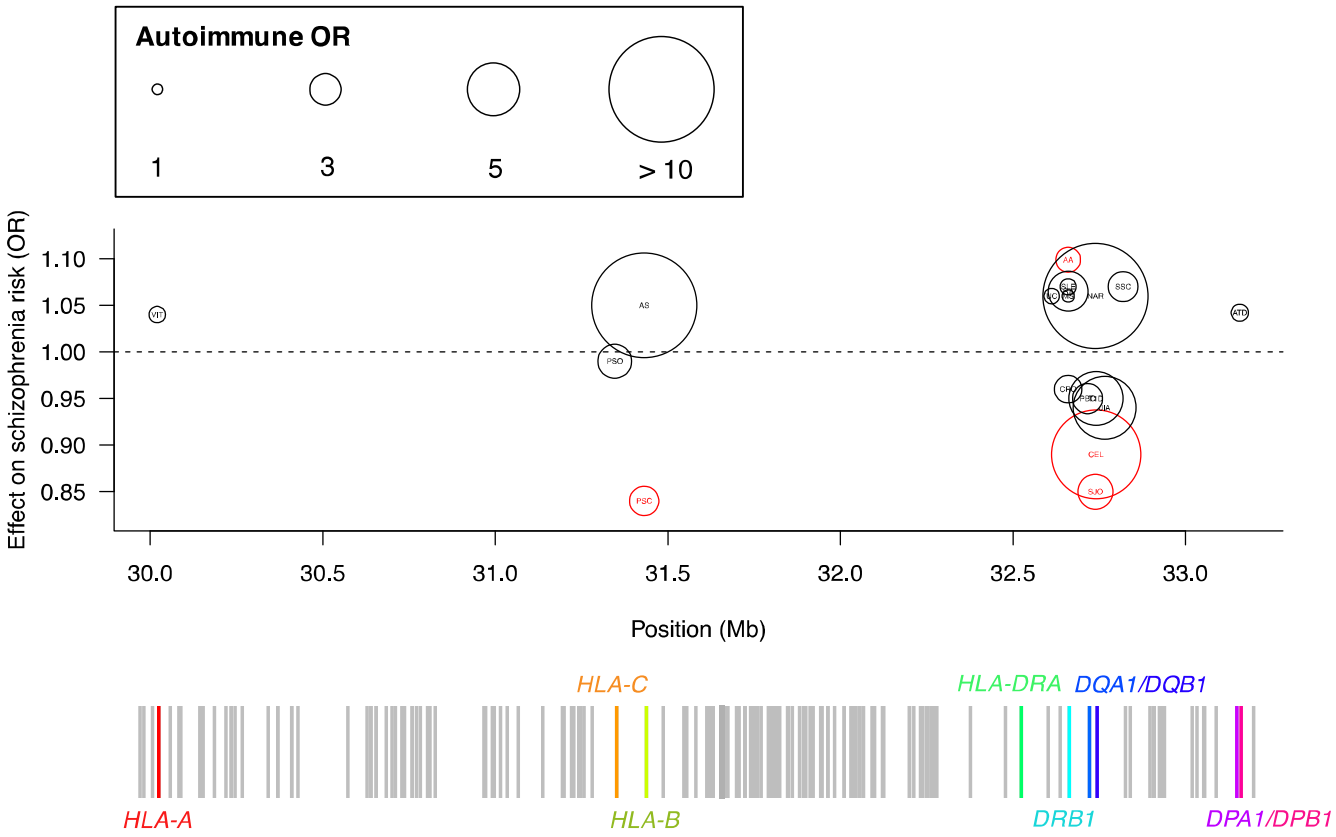

Figure 1. Identification of pleiotropic HLA variants

The most strongly associated HLA variant for each of the 19 immune-mediated diseases was evaluated for association with schizophrenia, using summary statistics from HLA imputation and association testing in the schizophrenia dataset as previously described.[9] x-axis corresponds to position in the classical HLA region, shown in megabase pairs (Mb); y-axis corresponds to the HLA variant's odds ratio (OR) in schizophrenia. Dashed line indicates OR=1. Size of the circle corresponds to the HLA variant's OR in the immune disease of interest, which is indicated in the circle center. Red circles indicate HLA variants associated with schizophrenia above the Bonferroni significance threshold ($p<8.6x10^{-5}$). Gene map below indicates location of coding HLA genes (coloured lines); gray lines correspond to non-HLA genes in the region. Disease abbreviations as defined in **Table 1**.

Using PRS, we observed that genetic liability for increased height protected against schizophrenia (β_{GRS}=-0.11, p=1.2x10^{-11} at p_T<1). The significant inverse association of height PRSs with schizophrenia case-status we observed may reflect the greater sensitivity of this approach to subtle population stratification, sample sharing, and/or true genetic overlap. Overall, our benchmarking indicated that the pleiotropy we detected between schizophrenia and immune disease was subtle.

To assess whether cryptic sample sharing between the immune and schizophrenia GWASs could be driving this modest pleiotropy, we conducted leave-half-out analyses. If the observed pleiotropy was driven by samples shared between certain schizophrenia cohorts and the immune disease GWASs, the PRS association should not be consistently observed across subsamples leaving out half of the schizophrenia cohorts (i.e. proportion of subsamples with PRS significantly associated with schizophrenia case-status ≤0.50). Across 1,000 subsamples (N_{cases} ranging from 3,985-13,074) leaving out a randomly selected 14 cohorts, we observed a high proportion of subsamples with PRSs significantly associated with schizophrenia (p<0.05 at p_T<1) for height (0.99), NAR (0.72), PBC (0.95), PSO (0.84), SLE (0.97), T1D (0.95), and UC (0.70) suggesting our findings were not driven by sample sharing. We also applied LDSC, a method robust to sample sharing,[29] using summary statistics from the 49 European-ancestry cohorts in the schizophrenia GWAS[1] (**Supplementary Table 5**). Notably, LDSC is less sensitive than PRS and is not robust when applied to genetic data obtained from specialty chips (e.g. Immunochip).[29] As expected, our positive control (bipolar disorder) showed significant genetic overlap with schizophrenia (r_g=0.75±0.05, p=8.5x10^{-60}) while our negative control (height) showed no such overlap (r_g=-0.004±0.02, p=0.84; **Supplementary Table 5**). LDSC confirmed significant genetic overlap with schizophrenia for PBC, PSO, SLE, and UC (r_g=0.10-0.18, see **Supplementary Table 5**) indicating the association of PRSs for these diseases was not driven by shared samples. We also observed significant genetic overlap with schizophrenia for NAR and T1D using LDSC, with the caveat that these datasets were genotyped using Immunochip and did not survive correction for the six tests performed (**Supplementary Table 5**). In agreement with our PRS results, the genetic correlations obtained using LDSC were modest, with r_g values about a fifth of that previously reported for bipolar disorder, and a quarter of that for major depressive disorder.[29]

Given the significant sex bias of autoimmune diseases, with women at greater risk overall,[39] we hypothesized that there may be sex-dependent pleiotropy between schizophrenia and some immune diseases. We therefore performed sex-stratified PRS. As expected, genetic scores for height showed significant association with schizophrenia in both males and females. Three of the immune diseases (PBC, PSO, T1D) with significant main effects showed sex-dependent effects, with greater signal among males (**Supplementary Table 4**). Additionally, although genetic scores for MS were not significantly associated with schizophrenia in the total sample there was significant association among males (R^2=0.03, p=1.26x10^{-3} at p_T<1; **Supplementary Table 4**). Given the greater statistical power for the male subset of the schizophrenia GWAS, we performed simulations by selecting random subsamples of male cases and controls equal in size to the female sample (5,321 cases and 9,094 controls).

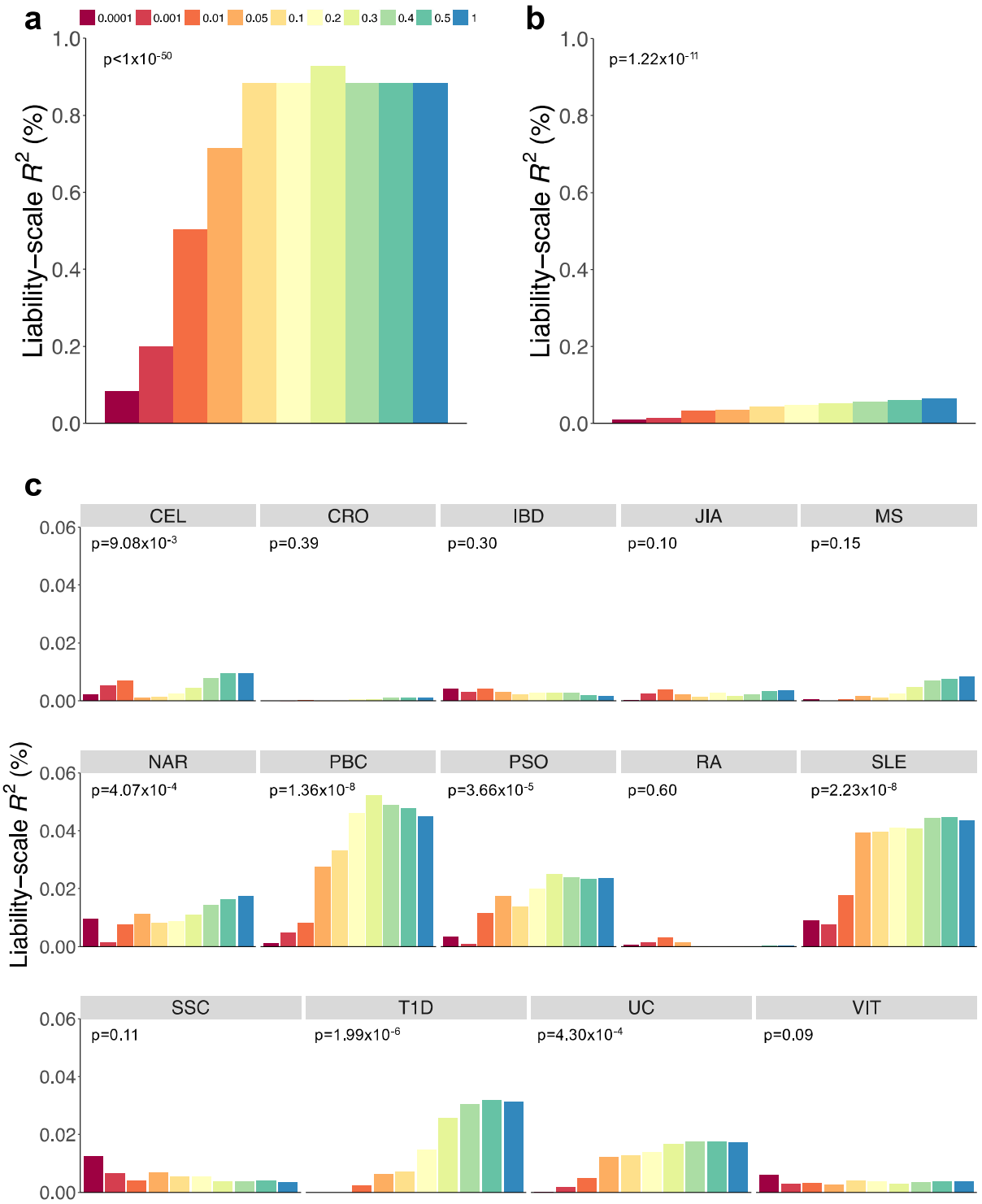

Figure 1. Prediction of schizophrenia using genetic liability for 14 immune-mediated diseases excluding HLA variants

Prediction of schizophrenia case-status using polygenic risk scores constructed for bipolar disorder, a positive control (a), human height, a negative control (b), and 14 immune-mediated diseases. Liability-scale R^2 is shown for PRSs derived using ten significance thresholds (p_T), represented by coloured bars. Significance estimates provided are for the least stringent $p_T<1$ threshold, which included all SNPs. For complete results across representative p_T, see **Supplementary Table 3**. Variance in schizophrenia case-

status explained is approximately 0.8% for bipolar disorder PRSs using previously published data[25]. Variance in schizophrenia case-status explained by human height risk scores is approximately 0.06%, while immune-mediated disease PRSs explained <0.06% of variance. Disease abbreviations as defined in Error! Reference source not found..

If the stronger pleiotropy between schizophrenia and MS, PBC, PSO, and T1D among males was driven by the larger sample size rather than a true sex-dependent effect, there should be no consistent association of PRSs with schizophrenia in these subsamples (i.e. proportion of subsamples with significant PRS ≤0.50). Across 1,000 subsamples, the proportion with significant PRS ($p<1.8x10^{-3}$ at $p_T<1$) was high for PBC (0.94) and T1D (0.87), suggesting our finding of a greater pleiotropic effect among males for these diseases was not driven solely by lower statistical power among females; this was not the case for PSO (0.59) or MS (0.21). Next, we performed formal statistical tests for an interaction between sex and genetic scores for these four immune diseases. We observed a nominally significant interaction for MS ($p<0.05$ at several p_T; **Supplementary Table 4**), noting that this finding did not survive correction for multiple testing. The remaining immune diseases did not show significant sex interactions, although the direction of effect was consistent with a greater pleiotropic effect in males (**Supplementary Table 4**).

Discussion

Using a variety of genetic approaches, we provide evidence of modest pleiotropy between schizophrenia and several immune diseases. Outside of the HLA region, we identified five SNPs with pleiotropic effects - influencing risk for both autoimmune disease and schizophrenia. Interestingly, the nearest genes for four of the pleiotropic variants have been implicated in calcium signaling, suggesting this may be a shared risk pathway.[25, 40] Parallel to its role in synaptic transmission, calcium signaling plays an important role in lymphocyte activation.[41] However, we note that two of the pleiotropic variants are reportedly eQTLs for more distant genes not necessarily involved in calcium signaling, and further work is required to confirm their biological function.

Within the HLA region, we identified four potentially pleiotropic variants. An important caveat is that, unlike the 19 immune diseases investigated, coding HLA variants do not appear to be the primary drivers of the association in this region in schizophrenia.[9, 42] Therefore, the biological significance of these particular HLA variants in schizophrenia is likely limited.

Using PRS we observed shared genetic liability with schizophrenia for six immune diseases (NAR, PBC, PSO, SLE, T1D, and UC), all of which have been previously reported to co-occur with schizophrenia.[3, 5, 43] Thus, currently available genetic data suggest that shared genetic risk may contribute to the co-occurrence of some immune diseases in schizophrenia - particularly PBC, PSO, SLE, and UC, which also showed robust genetic correlation with schizophrenia using LDSC. Possible explanations for this shared genetic risk include the presence of a hidden subgroup of “autoimmune-like” schizophrenia cases and/or sharing of specific biological pathways between schizophrenia and these particular immune diseases. Sample size was the strongest driver of statistical power, and it will be worthwhile to revisit these analyses as samples grow.

Importantly, PRSs for human height – analyzed as a negative control – showed stronger association with schizophrenia than any of the immune diseases. An inverse epidemiological relationship between height and schizophrenia has been reported,[27, 28]

consistent with our PRS findings. The reasons for the discrepancy between PRS and LDSC, which showed no genetic correlation between height and schizophrenia (as previously reported),[29] are unclear. One possibility is that PRS, which uses individual-level genotype data as opposed to summary statistics, is a more sensitive method to detect genome-wide pleiotropy. If this is the case, it raises a broader question regarding how much genetic overlap is expected across complex traits in general using the PRS approach. An alternative explanation that must be considered is that PRS may be more vulnerable to confounding by cryptic population stratification or sample sharing.

To our knowledge, this is the first time that sex-dependent pleiotropy with immune diseases has been investigated in schizophrenia. We found nominal evidence of male-specific pleiotropy for MS, and a stronger pleiotropic effect among males for PBC, PSO, and T1D although the latter were not statistically significant. Interestingly, animal studies indicate that sex hormones have opposing effects on predisposition to schizophrenia and autoimmunity; estrogen has been reported to protect against the development of schizophrenia,[44] while androgens appear to protect against the development autoimmune diseases.[45, 46] We emphasize that our sex-dependent findings require validation in independent samples. If replicated, one possibility is that sex hormones modulate pathogenesis among genetically vulnerable individuals, making males more likely to develop schizophrenia and females more likely to develop autoimmune diseases.

Our work adds to a growing body of literature suggesting pleiotropy exists between schizophrenia and autoimmune diseases. Genetic overlap with schizophrenia has been previously reported for CRO,[47, 48] MS,[49] RA (both positive[47] and negative[50] genetic correlations), T1D,[47] and UC[48] (see **Supplementary Table 6** for a summary of previous studies). Additionally, Tylee and Glatt have recently used LDSC to evaluate genetic correlations for a wide range of brain and immune phenotypes, finding nominally significant overlap between schizophrenia and CRO as well as UC (personal communication, July 28, 2016). Our results are consistent with previously reported pleiotropy between schizophrenia and both T1D and UC; while we did not observe pleiotropy between schizophrenia and MS in the total sample, there was a significant sex-dependent effect with pleiotropy among males. We provide new evidence of pleiotropy with NAR and PBC (not previously investigated) as well as PSO and SLE (previously reported to show no genetic overlap with schizophrenia[51]). The variances in schizophrenia case-status explained by PRSs for these immune diseases were modest, and for MS appeared to be sex-dependent, which may explain discrepant findings across studies using different methods and GWAS datasets. As genetic samples continue to grow, and our understanding of the degree of genetic overlap expected among complex traits evolves, we will be in a better position to evaluate the biological significance of the apparent pleiotropy between schizophrenia and autoimmune diseases.

URLs

LD Score database:
ftp://atguftp.mgh.harvard.edu/brendan/1k_eur_r2_hm3snps_se_weights.RDS

GWAS summary statistics:

- Celiac disease
  https://www.immunobase.org/downloads/protected_data/iChip_Data/hg19_gwas_ic_cel_trynka_4_19_1.tab.gz
- Crohn's disease, inflammatory bowel disease, ulcerative colitis
  ftp://ftp.sanger.ac.uk/pub/consortia/ibdgenetics/iibdgc-trans-ancestry-filtered-summary-stats.tgz
- Juvenile idiopathic arthritis
  https://www.immunobase.org/downloads/protected_data/iChip_Data/hg19_gwas_ic_jia_hinks_UK_4_19_1.tab.gz
- Multiple sclerosis
  https://www.immunobase.org/downloads/protected_data/GWAS_Data/hg19_gwas_ms_imsgc_4_19_1.tab.gz
- Narcolepsy
  https://www.immunobase.org/downloads/protected_data/iChip_Data/hg19_gwas_ic_nar_faraco_4_19_1.tab.gz
- Rheumatoid arthritis
  http://www.broadinstitute.org/ftp/pub/rheumatoid_arthritis/Stahl_etal_2010NG/
- Systemic lupus erythematosus
  https://www.immunobase.org/downloads/protected_data/GWAS_Data/hg19_gwas_sle_bentham_4_20_0.tab.gz
- Type 1 diabetes
  https://www.immunobase.org/downloads/protected_data/iChip_Data/hg19_gwas_ic_t1d_onengut_meta_4_19_1.tab.gz

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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