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Schizophrenia Working Grp Psychiat

2020-06-25

Schizophrenia Working Grp Psychiat , Kamitaki , N , Sekar , A , Handsaker , R E , McCarroll , S A , Eriksson , J , Palotie , A , Daly , M , Paunio , T & Pietiläinen , O 2020 , ' Complement genes contribute sex-biased vulnerability in diverse disorders ' , Nature , vol. 582 , no. 7813 , pp. 577-+ . https://doi.org/10.1038/s41586-020-2277-x

http://hdl.handle.net/10138/325153
https://doi.org/10.1038/s41586-020-2277-x

acceptedVersion

Published in final edited form as:

Nature. 2020 June ; 582(7813): 577–581. doi:10.1038/s41586-020-2277-x.

Complement genes contribute sex-biased vulnerability in diverse illnesses

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Author Contributions

N.K., A.S., T.J.V., and S.A.M. conceived the genetic studies. M.T.P., C.N.P., and M.B. collected and contributed whole-genome sequence data for the Genomic Psychiatry Cohort. R.E.H. and C.W.W. genotyped *C4* structural variation in the Genomic Psychiatry Cohort and optimized variant selection for use as a reference panel in the imputation of *C4* variation into lupus and schizophrenia cohorts (Extended Data Fig. 1). T.J.V., R.R.G., L.A.C., C.D.L., R.P.K., J.B.H., K.M.K., D.L.M., and P.T. contributed genotype data and imputation of non-*C4* variation for analysis of SLE cohorts. K.E.T. and L.A.C. contributed genotype and phenotype data along with imputation of non-*C4* variation for analysis of the SjS cohort. Investigators in the Schizophrenia Working Group of the Psychiatric Genomics Consortium collected and phenotyped cohorts and contributed genotype data for analysis of schizophrenia cohorts. N.K did the imputation and association analysis (Figs. 1, 2, 3a, b and Extended Data Figs. 2, 3, 4, 5, and 6). T.J.V., R.R.G., and D.L.M. provided valuable advice on the analysis and interpretation of SLE association results. R.A.O. and L.M.O.L collected and provided CSF samples composing the group from Utrecht, Netherlands. C.E.S. collected and provided CSF samples composing the Brigham & Women's Hospital group. H.d.R and K.T. performed the C4 and C3 immunoassay experiments on CSF samples (Fig. 3c, d and Extended Data Fig. 7a). N.K. did the analysis of plasma C4 and C3 concentrations (Extended Data Fig. 7b–f). S.A.M and N.K. wrote the manuscript with contributions from all authors.

Competing interests

The authors declare no competing interests.

Supplementary Information is available for this paper.

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Abstract

Many common illnesses differentially affect men and women for unknown reasons. The autoimmune diseases lupus and Sjögren's syndrome affect nine times more women than men[1], whereas schizophrenia affects men more frequently and severely[2]. All three illnesses have their strongest common genetic associations in the Major Histocompatibility Complex (MHC) locus, an association that in lupus and Sjögren's syndrome has long been thought to arise from alleles of the human leukocyte antigen (*HLA*) genes at that locus[3–6]. Here we show that the complement component 4 (*C4*) genes, which are also in the MHC locus and were recently found to increase risk for schizophrenia[7], generate 7-fold variation in risk for lupus (95% CI: 5.88–8.61; $p < 10^{-117}$ in total) and 16-fold variation in risk for Sjögren's syndrome (95% CI: 8.59–30.89; $p < 10^{-23}$ in total) among individuals with common *C4* genotypes, with *C4A* protecting more strongly than *C4B* in both illnesses. The same alleles that increase risk for schizophrenia greatly reduced risk for lupus and Sjögren's syndrome. In all three illnesses, *C4* alleles acted more strongly in men than in women: common combinations of *C4A* and *C4B* generated 14-fold variation in risk for lupus, 31-fold variation in risk for Sjögren's syndrome, and 1.7-fold variation in schizophrenia risk among men (vs. 6-fold, 15-fold, and 1.26-fold among women respectively). At a protein level, both C4 and its effector C3 were present at greater levels in men than women in cerebrospinal fluid ($p < 10^{-5}$ for both C4 and C3) and plasma[8,9] among adults ages 20–50, corresponding to the ages of differential disease vulnerability. Sex differences in complement protein levels may help explain the larger effects of *C4* alleles in men, women's greater risk of SLE and Sjögren's, and men's greater vulnerability in schizophrenia. These results implicate the complement system as a source of sexual dimorphism in vulnerability to diverse illnesses.

Systemic lupus erythematosus (SLE, or "lupus") is a systemic autoimmune disease of unknown cause. Risk of SLE is heritable (66%[10]), although SLE may have environmental triggers, as its onset often follows events that damage cells, such as infections and severe

sunburns[11]. Most SLE patients produce autoantibodies against nucleic acid complexes, including ribonucleoproteins and DNA[12].

In genetic studies, SLE associates most strongly with variation across the major histocompatibility complex (MHC) locus, which contains the human leukocyte antigen (*HLA*) genes[3]. However, conclusive attribution of this association to specific genes and alleles has been difficult; the identities of the most likely genetic sources have been frequently revised as genetic studies have grown in size[4,5]. In several other autoimmune diseases, including type 1 diabetes, celiac disease, and rheumatoid arthritis, strong effects of the MHC locus arise from *HLA* alleles that cause the peptide binding groove of HLA proteins to present a disease-critical autoantigen[13,14]. In SLE, by contrast, genetic variants in the MHC locus (including SNPs and *HLA* alleles) associate broadly with the presence of diverse autoantibodies[15].

The complement component 4 (*C4A* and *C4B*) genes are also present in the MHC genomic region, between the class I and class II *HLA* genes. Classical complement proteins help eliminate debris from dead and damaged cells, attenuating the visibility of diverse intracellular proteins to the adaptive immune system. *C4A* and *C4B* commonly vary in genomic copy number[16] and encode complement proteins with distinct affinities for molecular targets[17,18]. SLE frequently presents with hypocomplementemia that worsens during flares, possibly reflecting increased active consumption of complement[19]. Rare cases of severe, early-onset SLE can involve complete deficiency of a complement component (C4, C2, or C1Q)[20,21], and one of the strongest common-variant associations in SLE maps to *ITGAM*, which encodes a receptor for C3, the effector of C4[22]. Although total *C4* gene copy number associates with SLE risk[23,24], this association is thought to arise from linkage disequilibrium (LD) with alleles of nearby *HLA* genes[25], which have been the focus of fine-mapping analyses[3,4].

The complex genetic variation at *C4* – arising from many alleles with different numbers of *C4A* and *C4B* genes – has been challenging to analyze in large cohorts. A recently feasible approach to this problem is based on imputation: people share long haplotypes with the same combinations of SNP and *C4* alleles, such that *C4A* and *C4B* gene copy numbers can be imputed from SNP data[7]. To analyze *C4* in large cohorts, we developed a way to identify *C4* alleles from whole-genome sequence (WGS) data (Extended Data Fig. 1a, b), then analyzed WGS data from 1,265 individuals (from the Genomic Psychiatry Cohort[26,27]) to create a large multi-ancestry panel of 2,530 reference haplotypes of MHC-region SNPs and *C4* alleles (Extended Data Fig. 1c) – ten times more than in earlier work[7]. We then analyzed SNP data from the largest SLE genetic association study[3] (ImmunoChip 6,748 SLE cases and 11,516 controls of European ancestry) (Extended Data Fig. 2a, b), imputing *C4* alleles to estimate the SLE risk associated with common combinations of *C4A* and *C4B* gene copy numbers (Fig. 1a).

Groups of research participants with the eleven most common combinations of *C4A* and *C4B* gene copy number exhibited 7-fold variation in their relative risk of SLE (Fig. 1a, Extended Data Fig. 2c). The relationship between SLE risk and *C4* gene copy number exhibited consistent, logical patterns across the 11 genotype groups. For each *C4B* copy

number, greater *C4A* copy number associated with reduced SLE risk (Fig. 1a, Extended Data Fig. 2c). For each *C4A* copy number, greater *C4B* copy number associated with more modestly reduced risk (Fig. 1a). Logistic-regression analysis estimated that the protection afforded by each copy of *C4A* (OR: 0.54; 95% CI: [0.51, 0.57]) was equivalent to that of 2.3 copies of *C4B* (OR: 0.77; 95% CI: [0.71,0.82]). We calculated an initial *C4*-derived risk score as 2.3 times the number of *C4A* genes, plus the number of *C4B* genes, in an individual's genome. Despite clear limitations of this risk score – it is imperfectly imputed from flanking SNP haplotypes (r^2 = 0.77, Extended Data Table 1) and only approximates *C4*-derived risk by using a simple, linear model (to avoid over-fitting the genetic data) – SNPs across the MHC genomic region tended to associate with SLE in proportion to their level of LD with this risk score (Extended Data Fig. 3a).

Combinations of many different *C4* alleles generate the observed variation in *C4A* and *C4B* gene copy number; particular *C4A* and *C4B* gene copy numbers have also arisen recurrently on multiple SNP haplotypes[7] (Extended Data Fig. 1c). Analysis of SLE risk in relation to each of these *C4* alleles and SNP haplotypes reinforced the conclusion that *C4A* contributes strong protection, and *C4B* more modest protection, from SLE, and that *C4* genes (rather than nearby variants) are the principal drivers of this variation in risk levels (Fig. 1b).

These results prompted us to consider whether other autoimmune disorders with similar patterns of genetic association at the MHC genomic region might also be driven in part by *C4* variation. Primary Sjögren's syndrome (SjS) is a heritable (54%[28]) systemic autoimmune disorder of exocrine glands, characterized primarily by dry eyes and mouth with other systemic effects. At a protein level, SjS is (like SLE) characterized by diverse autoantibodies, including antinuclear antibodies targeting ribonucleoproteins[29], and hypocomplementemia[30]. The largest source of common genetic risk for SjS lies in the MHC genomic locus[31], with associations to the same haplotype(s) as in SLE[6] and with heterogeneous *HLA* associations in different ancestries[32]. We imputed *C4* alleles into existing SNP data from a European-ancestry SjS case-control cohort (673 cases and 1153 controls). As in SLE, logistic-regression analyses found both *C4A* copy number (OR: 0.41; 95% CI: [0.34, 0.49]) and *C4B* copy number (OR: 0.67; 95% CI: [0.53, 0.86]) to be protective against SjS. The risk-equivalent ratio of *C4B* to *C4A* gene copies was similar in SjS and SLE (about 2.3 to 1); also, as with SLE, nearby SNPs associated with SjS in proportion to their LD with a *C4*-derived risk score ((2.3)*C4A*+*C4B*) (Extended Data Fig. 3b). The distribution of SjS risk across the individual *C4* alleles and haplotypes revealed a pattern that, as in SLE, supported greater protective effect from *C4A* than *C4B,* and little effect of flanking SNP haplotypes (Fig. 1b).

The association of SLE and SjS with *C4* gene copy number has long been attributed to the *HLA-DRB1*03:01* allele. In European populations, *DRB1*03:01* is in strong LD (r^2 = 0.71) with the common *C4*-B(S) allele, which lacks any *C4A* gene and is the highest-risk *C4* allele in our analysis (Fig. 1b); many MHC-region SNPs associated with SLE and SjS in proportion to their LD correlations with both *C4* and *DRB1**03:01 (Extended Data Fig. 4a, b). Cohorts with other ancestries can have recombinant haplotypes that disambiguate the contributions of alleles that are in LD in Europeans. Among African Americans, we found that common *C4* alleles exhibited far less LD with *HLA* alleles; in particular, the LD

between *C4*-B(S) and *DRB1**03:01 was low ($r^2 = 0.10$) (Extended Data Table 2). Thus, genetic data from an African American SLE cohort (1,494 cases, 5,908 controls) made it possible to distinguish between these potential genetic effects. Joint association analysis of *C4A*, *C4B*, and *DRB1*0301* implicated *C4A* ($p < 10^{-14}$) and *C4B* ($p < 10^{-5}$) but not *DRB1*0301* ($p = 0.29$) (Extended Data Table 3). Each *C4* allele associated with effect sizes of similar magnitude on SLE risk in Europeans and African Americans (Fig. 2a). An analysis specifically of combinations of *C4*-B(S) and *DRB1**03:01 allele dosages in African Americans showed that *C4*-B(S) alleles consistently increased SLE risk regardless of *DRB1**03:01 status, whereas *DRB1**03:01 had no consistent effect when controlling for *C4*-B(S) (Fig. 2b). Although *C4* alleles had less LD with nearby variants on African American than on European haplotypes, SNPs across the genomic region associated with SLE in proportion to LD correlations with *C4* in African Americans as well (Extended Data Fig. 4c).

Accounting for *C4* alleles in jointly analyzing the SLE association data from African American and European ancestry cohorts also enabled the mapping of an additional, more-modest genetic effect independent of *C4*; this effect (tagged by rs2105898 and rs9271513) appeared to involve noncoding variation in the *HLA* class II XL9 region that associates most strongly with expression levels (rather than the coding sequence) of many *HLA* class II genes (Extended Data Figs. 3c, d, 4d–l, 5, and Supplementary Note 1).

Alleles at *C4* that increase dosage of *C4A* (and to a more modest extent *C4B*) appear to protect strongly against SLE and SjS (Fig. 1a, b); by contrast, alleles that increase expression of *C4A* in the brain are more common among research participants with schizophrenia[6]. These same illnesses exhibit striking, and opposite, sex differences: SLE and SjS are nine times more common among women of childbearing age than among men of a similar age[1], whereas in schizophrenia, women exhibit less severe symptoms, more frequent remission of symptoms, lower relapse rates, and lower overall incidence[2]. Though the vast majority of genetic associations in complex diseases are shared between men and women[33], the SNPs that most strongly associate with SLE risk within the MHC region associate to larger potential effect sizes in men[34]. Hence, we sought to evaluate the possibility that the effects of *C4* alleles on risk in SLE, SjS, and schizophrenia might differ between men and women.

Analysis indicated that the effects of *C4* alleles were stronger in men. When a sex-by-*C4* interaction term was included in association analyses, this term was significant for both SLE ($p = 0.002$) and schizophrenia ($p = 0.0024$), with larger *C4* effects in men for both disorders. (Analysis of SjS had limited power due to the small number of men affected by SjS.) For both SLE and schizophrenia, the individual *C4* alleles consistently associated with stronger effects in men than women (Fig. 3a, b). SNPs across the MHC genomic region exhibited sex-biased association to SLE, SjS, and schizophrenia to the extent of their LD with *C4* (Extended Data Fig. 6a–c).

The stronger effects of *C4* alleles on male relative to female risk could arise from sex differences in *C4* RNA expression, C4 protein levels, or downstream responses to C4. Analysis of RNA expression in human tissues, using data from GTEx[35], identified no sex

differences in *C4* RNA expression in brain, blood, liver, or lymphoblastoid cells (a more-detailed description of this analysis can be found in Supplementary Note 2). We then analyzed C4 protein in cerebrospinal fluid (CSF) from two panels of adult research participants (n = 589 total) in whom we had also measured *C4* gene copy number (by direct genotyping or imputation). CSF C4 protein levels correlated strongly with *C4* gene copy number ($p < 10^{-10}$, Extended Data Fig. 7a), so we normalized C4 protein measurements to the number of *C4* gene copies. CSF from adult men contained on average 27% more C4 protein per *C4* gene copy than CSF from women (meta-analysis $p = 9.9 \times 10^{-6}$, Fig. 3c). C4 acts by activating the complement component 3 (C3) protein, promoting C3 deposition onto targets in tissues. CSF levels of C3 protein were also on average 42% higher among men than women (meta-analysis $p = 7.5 \times 10^{-7}$, Fig. 3d).

The elevated concentrations of C3 and C4 proteins in CSF of men parallel earlier findings that, in plasma, C3 and C4 are also present at higher levels in men than women[8,9]. The large sample size (n > 50,000) of the plasma studies allows sex differences to be further analyzed as a function of age. Both men and women undergo age-dependent elevation of C4 and C3 levels in plasma, but this occurs early in adulthood (age 20–30) in men and closer to menopause (age 40–50) in women, with the result that male–female differences in complement protein levels are observed primarily during the reproductive years (ages 20–50)[8,9]. We replicated these findings using measurements of C3 and gene copy number-corrected C4 protein in plasma from adults, finding (as in the earlier plasma studies[8,9] and in CSF, Fig. 3c, d) that these differences are most pronounced during the reproductively active years of adulthood (ages 20–50) (Extended Data Fig. 7b–d). We also observed that SjS patients have lower C4 serum levels than controls ($p < 1{\times}10^{-20}$, Extended Data Fig. 7e) even after correcting for *C4* gene copy number ($p < 1{\times}10^{-8}$, Extended Data Fig. 7f), suggesting that hypocomplementemia in SjS is not simply due to *C4* genetics but also reflects disease effects on ambient complement levels, for example due to complement consumption. The ages of pronounced sex difference in complement levels corresponded to the ages at which men and women differ in disease incidence: in schizophrenia, men outnumber women among cases incident in early adulthood, but not among cases incident after age 40[2]; in SLE, women greatly outnumber men among cases incident during the child-bearing years, but not among cases incident after age 50 or during childhood[36]; in SjS, the large relative vulnerability of women declines in magnitude after age 50[37].

Our results indicate that the MHC genomic region shapes vulnerability in lupus and SjS – two of the three most common rheumatic autoimmune diseases – in a very different way than in type I diabetes, rheumatoid arthritis, and celiac disease. In the latter diseases, precise interactions between specific *HLA* protein variants and specific autoantigens determine risk [13,14]. In SLE and SjS, however, the genetic variation implicated here points instead to the continuous, chronic interaction of the immune system with very many potential autoantigens. Because complement facilitates the rapid clearance of debris from dead and injured cells, elevated levels of C4 protein likely attenuate interactions between the adaptive immune system and ribonuclear self-antigens at sites of cell injury, pre-empting the development of autoimmunity. The additional *C4*-independent genetic risk effect described here (associated with rs2105898) may also affect autoimmunity broadly, rather than antigen-specifically, by regulating expression of many HLA class II genes (including *DRB1, DQA1,*

and *DQB1*). Mouse models of SLE indicate that once tolerance is broken for one self-antigen, autoreactive germinal centers generate B cells targeting other self-antigens[38]; such "epitope spreading" could lead to autoreactivity against many related autoantigens, regardless of which antigen(s) are involved in the earliest interactions with immune cells. Further supporting such a model, higher copy number of *C4* associates with lower risk of AQP4-IgG-seropositive neuromyelitis optica (NMO-IgG+)[39], in which seropositive patients have increased incidence of other non-organ-specific autoantibodies such as those seen in SLE and SjS[40]. B-cells also express the complement receptors CR1 and CR2[41], providing an additional candidate mechanism for regulation by C4 and C3.

We note that the role of complement proteins in preventing the emergence of autoimmunity may be very different than their (potentially disease-exacerbating) role once autoimmunity has been established. Also, our genetic findings address the development of SLE and SjS rather than complications that arise in any specific organ. A few percent of SLE patients develop neurological complications that can include psychosis[42]; though psychosis is also a symptom of schizophrenia, neurological complications of SLE do not resemble schizophrenia more broadly, and likely have a different etiology.

The same *C4* alleles that increase vulnerability to schizophrenia appeared to protect strongly against SLE and SjS. This pleiotropy will be important to consider in efforts to engage the complement system therapeutically. The complement system contributed to these pleiotropic effects more strongly in men than in women. Moreover, though the natural allelic series at *C4* allowed human-genetic analysis to establish dose-risk relationships for *C4* in men and women, sexual dimorphism in the complement-protein levels also included complement component 3 (C3). Why and how biology has come to create this sexual dimorphism in the complement system in humans presents interesting questions for immune and evolutionary biology.

Methods

Creation of a *C4* reference panel from whole-genome sequence data

We constructed a reference panel for imputation of *C4* structural haplotypes using whole-genome sequencing data for 1265 individuals from the Genomic Psychiatry Cohort[26]. The reference panel included individuals of diverse ancestry, including 765 Europeans, 250 African Americans, and 250 people of reported Latino ancestry.

We estimated the diploid *C4* copy number, and separately the diploid copy number of the contained HERV segment, using Genome STRiP[44]. Briefly, Genome STRiP carefully calibrates measurements of read depth across specific genomic segments of interest by estimating and normalizing away sample-specific technical effects such as the effect of GC content on read depth (estimated from the genome-wide data). To estimate *C4* copy number, we genotyped the segments 6:31948358–31981050 and 6:31981096–32013904 (hg19) for total copy number, but masked the intronic HERV segments that distinguish short (S) from long (L) *C4* gene isotypes. For the HERV region, we genotyped segments 6:31952461–31958829 and 6:31985199–31991567 (hg19) for total copy number. Across the 1,265 individuals, the resultant locus-specific copy-number estimates exhibited a strongly multi-

modal distribution (Extended Data Fig. 1a) from which individuals' total *C4* copy numbers could be readily inferred.

We then estimated the ratio of *C4A* to *C4B* genes in each individual genome. To do this, we extracted reads mapping to the paralogous sequence variants that distinguish *C4A* from *C4B* (hg19 coordinates 6:31963859–31963876 and 6:31996597–31996614) in each individual, combining reads across the two sites. We included only reads that aligned to one of these segments in its entirety. We then counted the number of reads matching the canonical active site sequences for *C4A* (CCC TGT CCA GTG TTA GAC) and *C4B* (CTC TCT CCA GTG ATA CAT). We combined these counts with the likelihood estimates of diploid *C4* copy number (from Genome STRiP) to determine the maximum likelihood combination of *C4A* and *C4B* in each individual (Extended Data Fig. 1b). We estimated the genotype quality of the *C4A* and *C4B* estimate from the likelihood ratio between the most likely and second most likely combinations.

To phase the *C4* haplotypes, we first used the GenerateHaploidCNVGenotypes utility in Genome STRiP to estimate haplotype-specific copy-number likelihoods for *C4* (total *C4* gene copy number), *C4A*, *C4B*, and HERV using the diploid likelihoods from the prior step as input. Default parameters for GenerateHaploidCNVGenotypes were used, plus -genotypeLikelihoodThreshold 0.0001. The output was then processed by the GenerateCNVHaplotypes utility in Genome STRiP to combine the multiple estimates into likelihood estimates for a set of unified structural alleles. GenerateCNVHaplotypes was run with default parameters, plus -defaultLogLikelihood −50, -unknownHaplotypeLikelihood −50, and -sampleHaplotypePriorLikelihood 2.0. The resultant VCF was phased using Beagle 4.1 (beagle_4.1_27Jul16.86a) in two steps: first, performing genotype refinement from the genotype likelihoods using the Beagle gtgl= and maxlr=1000000 parameters, and then running Beagle again on the output file using gt= to complete the phasing.

Our previous work suggested that several *C4* structures segregate on different haplotypes, and probably arose by recurrent mutation on different haplotype backgrounds[7]. The GenerateCNVHaplotypes utility requires as input an enumerated set of structural alleles to assign to the samples in the reference cohort, including any structurally equivalent alleles, with distinct labels to mark them as independent, plus a list of samples to assign (with high likelihood) to specific labeled input alleles to disambiguate among these recurrent alleles. The selection of the set of structural alleles to be modeled, along with the labeling strategy, is important to our methodology and the performance of the reference panel. In the reference panel, each input allele represents a specific copy number structure and optionally includes a label that differentiates the allele from other independent alleles with equivalent structure. We use the notation <H_n_n_n_n_L> to identify each allele, where the four integers following the H are, respectively, the (redundant) haploid count of the total number of *C4* copies, *C4A* copies, *C4B* copies and HERV copies on the haplotype. For example, <H_2_1_1_1> was used to represent the "AL-BS" haplotype. The optional final label L is used to distinguish potentially recurrent haplotypes with otherwise equivalent structures (under the model) that should be treated as independent alleles for phasing and imputation.

To build the reference panel, we experimentally evaluated a large number of potential sets of structural alleles and methods for assigning labels to potentially recurrent alleles. For each evaluation, we built a reference panel using the 1265 reference samples, and then evaluated the performance of the panel via cross-validation, leaving out 10 different samples in each trial (5 samples in the last trial) and imputing the missing samples from the remaining samples in the panel. The imputed results for all 1265 samples were then compared to the original diploid copy number estimates to evaluate the performance of each candidate reference panel (Extended Data Table 1).

Using this procedure, we selected a final panel for downstream analysis that used a set of 29 structural alleles representing 16 distinct allelic structures (as listed in the reference panel VCF file). Each allele contained from one to three copies of *C4*. Three allelic structures (AL-BS, AL-BL, and AL-AL) were represented as a set of independently labeled alleles with 9, 3, and 4 labels, respectively.

To identify the number of labels to use on the different alleles and the samples to "seed" the alleles, we generated "spider plots" of the *C4* locus based on initial phasing experiments run without labeled alleles, and then clustered the resulting haplotypes in two dimensions based on the Y-coordinate distance between the haplotypes on the left and right sides of the spider plot. Clustering was based on visualizing the clusters (Extended Data Fig. 1c) and then manually choosing both the number of clusters (labels) to assign and a set of confidently assigned haplotypes to use to "seed" the clusters in GenerateCNVHaplotypes. This procedure was iterated multiple times using cross-validation, as described above, to evaluate the imputation performance of each candidate labeling strategy.

Within the data set used to build the reference panel, there is evidence for individuals carrying seven or more diploid copies of *C4*, which implies the existence of (rare) alleles with four or more copies of *C4*. In our experiments, attempting to add additional haplotypes to model these rare four-copy alleles reduced overall imputation performance. Consequently, we conducted all downstream analyses using a reference panel that models only alleles with up to three copies of *C4*. In the future, larger reference panels might benefit from modeling these rare four-copy alleles.

The reference panel will be available in dbGaP (accession # pending) with broad permission for research use.

Genetic data for SLE

For analysis of systemic lupus erythematosus (SLE), collection and genotyping of the European-ancestry cohort (6,748 cases, 11,516 controls, genotyped by ImmunoChip) as previously described[3]. Collection and genotyping of the African-American cohort (1,494 cases, 5,908 controls, genotyped by OmniExpress) as previously described[5].

Genetic data for SjS

For analysis of Sjögren's syndrome (SjS), collection and genotyping of the European-ancestry cohort (673 cases, 1,153 controls, genotyped by Omni2.5) as previously described[32] and available in dbGaP under study accession number phs000672.v1.p1.

Genetic data for schizophrenia

The schizophrenia analysis made use of genotype data from 40 cohorts of European ancestry (28,799 cases, 35,986 controls) made available by the Psychiatric Genetics Consortium (PGC) as previously described[43]. Genotyping chips used for each cohort are listed in Supplementary Table 3 of that study.

Imputation of *C4* alleles

The reference haplotypes described above were used to extend the SLE, SjS, or schizophrenia cohort SNP genotypes by imputation. SNP data in VCF format were used as input for Beagle v4.1[45,46] for imputation of *C4* as a multi-allelic variant. Within the Beagle pipeline, the reference panel was first converted to bref format. From the cohort SNP genotypes, we used only those SNPs from the MHC region (chr6:24–34 Mb on hg19) that were also in the haplotype reference panel. We used the conform-gt tool to perform strand-flipping and filtering of specific SNPs for which strand remained ambiguous. Beagle was run using default parameters with two key exceptions: we used the GRCh37 PLINK recombination map, and we set the output to include genotype probability (i.e., GP field in VCF) for correct downstream probabilistic estimation of *C4A* and *C4B* joint dosages.

Imputation of *HLA* alleles

For *HLA* allele imputation, sample genotypes were used as input for the R package HIBAG[47]. For both European ancestry and African American cohorts, publicly available multi-ethnic reference panels generated for the most appropriate genotyping chip (i.e. Immunochip for European ancestry SLE cohort, Omni 2.5 for European ancestry SjS cohort, and OmniExpress for African American SLE cohort) were used[48]. Default parameters were used for all settings. All class I and class II *HLA* genes were imputed. Output haplotype posterior probabilities were summed per allele to yield diploid dosages for each individual.

Associating single and joint *C4* structural allele dosages to SLE and SjS in European ancestry individuals

The analysis described above yields dosage estimates for each of the common *C4* structural haplotypes (e.g., AL-BS, AL-AL, etc.) for each genome in each cohort. In addition to performing association analysis on these structures (Fig 1b), we also performed association analysis on the dosages of each underlying *C4* gene isotype (i.e. *C4A*, *C4B*, *C4L*, and *C4S*). These dosages were computed from the allelic dosage (DS) field of the imputation output VCF simply by multiplying the dosage of a *C4* structural haplotype by the number of copies of each *C4* isotype that haplotype contains (e.g., AL-BL contains one *C4A* gene and one *C4B* gene).

C4 isotype dosages were then tested for disease association by logistic regression, with the inclusion of four available ancestry covariates derived from genome-wide principal component analysis (PCA) as additional independent variables, PC_c,

$$\text{logit}(\theta) \sim \beta_0 + \beta_1 C4 + \Sigma_c \beta_c PC_c + \varepsilon \quad (1)$$

where θ=E[SLE|**X**]. For SjS, the model instead included two available multiethnic ancestry covariates from dbGaP that correlated strongly with European-specific ancestry covariates (specifically, PC5 and PC7) and smoking status as independent variables. Coefficients for relative weighting of *C4A* and *C4B* dosages were obtained from a joint logistic regression,

$$\text{logit}(\theta) \sim \beta_0 + \beta_1 C4A + \beta_2 C4B + \Sigma_c \beta_c \text{PC}_c + \varepsilon \quad (2)$$

The values per individual of $\beta_1 C4A + \beta_2 C4B$ were used as a combined *C4* risk term for estimating both association strength (Extended Data Fig. 3a, b) as well as evaluating the relationship between the strength of nearby variants' association with SLE or SjS and linkage with *C4* variation (Extended Data Fig. 4a–c).

Joint dosages of *C4A* and *C4B* for each individual in the same cohort were estimated by summing across their genotype probabilities of paired structural alleles that encode for the same diploid copy numbers of both *C4A* and *C4B* (Extended Data Fig. 2a, b). For each individual/genome, this yields a joint dosage distribution of *C4A* and *C4B* gene copy number, reflecting any possible imputed haplotype-level dosages with nonzero probability. Joint dosages for *C4A* and *C4B* diploid copy numbers were tested for association with SLE in a joint model with the same ancestry covariates (Fig. 1a),

$$\text{logit}(\theta) \sim \beta_0 + \Sigma_{i,j} \beta_{i,j} \text{P}(C4A = i, C4B = j) + \Sigma_c \beta_c \text{PC}_c + \varepsilon \quad (3)$$

Calculation of composite *C4* risk for SLE

Because SLE risk strongly associated with *C4A* and *C4B* copy numbers (Fig. 1a) in a manner that can be approximated as – but is not necessarily linear or independent – a composite *C4* risk score was derived by taking the weighted sum of joint *C4A* and *C4B* dosages multiplied by the corresponding effect sizes from the aforementioned model of the joint *C4A* and *C4B* diploid copy numbers. The weights for calculating this composite *C4* risk term were computed from the data from the European ancestry cohort, and then applied unchanged to analysis of the African American cohort.

Associations of variants across the MHC region to SLE and SjS

Genotypes for non-array SNPs were imputed with IMPUTE2 using the 1000 Genomes reference panel; separate analyses were performed for the European-ancestry and African American cohorts. Unless otherwise stated, all subsequent SLE analyses were performed identically for both European ancestry and African American cohorts. Dosage of each variant, v_i, was tested for association with SLE or SjS in a logistic regression including available ancestry covariates (and smoking status for SjS) first alone (Extended Data Fig. 3a, b),

$$\text{logit}(\theta) \sim \beta_0 + \beta_1 v_i + \Sigma_c \beta_c \text{PC}_c + \varepsilon \quad (4)$$

then with *C4* composite risk (Extended Data Fig. 3c),

$$\text{logit}(\theta) \sim \beta_0 + \beta_1 v_i + \beta_2 C4 + \Sigma_c \beta_c PC_c + \varepsilon \quad (5)$$

where θ=E[SLE|**X**]. For SjS, the simpler weighted (2.3)*C4A*+*C4B* model was used instead of composite risk term, as the cohort's size gave poor precision to estimates of risk for many joint (*C4A*, *C4B*) copy numbers (Extended Data Fig. 3d). The Pearson correlation between the *C4* composite risk term and each other variant was computed and squared (r^2) to yield a measure of linkage disequilibrium between *C4* composite risk and that variant in that cohort.

Association analyses for specific *C4* structural alleles

The *C4* structural haplotypes were tested for association with disease (Fig. 1b, 2a) in a joint logistic regression that included (i) terms for dosages of the five most common *C4* structural haplotypes (AL-BS, AL-BL, AL-AL, BS, and AL), (ii) (for SLE and SjS) rs2105898 genotype, and (iii) ancestry covariates and (for SjS) smoking status,

$$\text{logit}(\theta) \sim \beta_0 + \beta_1 BS + \beta_2 AL + \beta_3 ALBS + \beta_4 ALBL + \beta_5 ALAL + \beta_6 rs2105898 + \Sigma_c \beta_c PC_c + \varepsilon \quad (6)$$

where θ=E[SLE|**X**]. Several of these common *C4* structural alleles arose multiple times on distinct haplotypes; we term the set of haplotypes in which such a common allele appeared as "haplogroups". The haplogroups can be further tested in a logistic regression model in which the structural allele appearing in all member haplotypes is instead encoded as dosages for each of the SNP haplotypes in which it appears. These association analyses (Fig. 1b, 2a) were performed as in (6), with structural allele dosages for ALBS, ALBL, and ALAL replaced by multiple terms for each distinct haplotype.

To delineate the relationship between *C4*-BS and *DRB1**03:01 alleles – which are highly linked in European ancestry haplotypes – allelic dosages per individual in the African American SLE cohort were rounded to yield the most likely integer dosage for each. Although genotype dosages for each are reported by BEAGLE and HIBAG respectively, probabilities per haplotype are not linked and multiplying possible diploid dosages could yield incorrect non-zero joint dosages. Joint genotypes were tested as individual terms in a logistic regression model (Fig. 2b),

$$\text{logit}(\theta) \sim \beta_0 + \Sigma_{i,j} \beta_{i,j} P(C4 - BS = i, DRB1*03{:}01 = j) + \Sigma_c \beta_c PC_c + \varepsilon \quad (7)$$

Sex-stratified associations of *C4* structural alleles and other variants with SLE, SjS, and schizophrenia

Determination of an effect from sex on the contribution of overall *C4* variation to risk for each disorder was done by including an interaction term between sex and *C4*; ie. (2.3)C4A +C4B for SLE and SjS and estimated C4A expression for schizophrenia:

$$\text{logit}(\theta) \sim \beta_0 + \beta_2 C4 + \beta_3 I_{Sex} + \beta_4 I_{Sex} C4 + \Sigma_c \beta_c PC_c + \varepsilon \quad (8)$$

Each variant in the MHC region was tested for association with among European ancestry cases and cohorts in a logistic regression as in models (4)–(6) using only male cases and controls, and then separately using only female cases and controls (Extended Data Fig. 6a–c). Likewise, allelic series analyses were performed as in (7), but in separate models for men and women (Fig. 3a, b).

To assess the relationship between sex bias in the risk associated with a variant and linkage to *C4* composite risk (as non-negative r^2), male and female log-odds were multiplied by the sign of the Pearson correlation between that variant and *C4* composite risk before taking the difference.

Analyses of cerebrospinal fluid

Cerebrospinal fluid (CSF) from healthy individuals was obtained from two research panels. The first panel, consisting of 533 donors (327 male, 126 female) from hospitals around Utrecht, Netherlands, was described previously[49,50]. The donors were generally healthy research participants undergoing spinal anesthesia for minor elective surgery. The same donors were previously genotyped using the Illumina Omni SNP array. To estimate *C4* copy numbers, we used SNPs from the MHC region (chr6:24–34 Mb on hg19) as input for *C4* allele imputation with Beagle, as described above in Imputation of *C4* alleles.

The second CSF panel sampled specimens from 56 donors (14 male, 42 female) from Brigham and Women's Hospital (BWH; Boston, MA, USA) under a protocol approved by the institutional review board at BWH (IRB protocol ID no. 1999P010911) with informed consent. These samples were originally obtained to exclude the possibility of infection, and clinical analyses had revealed no evidence of infection. Donors ranged in age from 18 to 64 years old. Blood samples from the same individuals were used for extraction of genomic DNA, and *C4* gene copy number was measured by droplet digital PCR (ddPCR) as previously described[7]. Samples were excluded from measurements if they lacked *C4* genotypes, sex information, or contained visible blood contamination.

C4 measurements were performed by sandwich ELISA of 1:400 dilutions of the original CSF sample using goat anti-sera against human C4 as the capture antibody (Quidel, A305, used at 1:1000 dilution), FITC-conjugated polyclonal rabbit anti-human C4c as the detection antibody (Dako, F016902–2, used at 1:3000 dilution), and alkaline phosphatase–conjugated polyclonal goat anti-rabbit IgG as the secondary antibody (Abcam, ab97048, used at 1:5000 dilution). C3 measurements were performed using the human complement C3 ELISA kit (Abcam, ab108823).

Because *C4* gene copy number had a large and proportional effect on C4 protein concentration in these CSF samples (Extended Data Fig. 7a), we corrected for *C4* gene copy number in our analysis of relationship between sex and C4 protein concentration, by normalizing the ratio of C4 protein (in CSF) to *C4* gene copies (in genome). Therefore, these analyses included only samples for which DNA was available or *C4* was successfully imputed. In total, 495 (332 male, 163 female) C4 and 304 (179 male, 125 female) C3 concentrations were obtained across both cohorts. Log-concentrations of C3 (ng/mL) and C4

(ng/[mL, per *C4* gene copy number]) protein were then used separately in linear regression models to estimate a sex-unbiased cohort-specific offset for each protein,

$$\log_{10}(\text{C3 or C4 concentration}) \sim \beta_0 + \beta_1 I_{\text{male}} + \beta_2 I_{\text{cohort}} + \varepsilon \quad (9)$$

to be applied to all concentrations for that protein. Estimation of average measurements by age for each sex was done by local polynomial regression smoothing (LOESS) (Fig. 3c, d). To evaluate the significance of sex effects, we used these cohort-corrected concentrations estimates and analyzed them with the non-parametric unsigned Mann-Whitney rank–sum test comparing concentration distributions for males and females.

Analyses of blood plasma

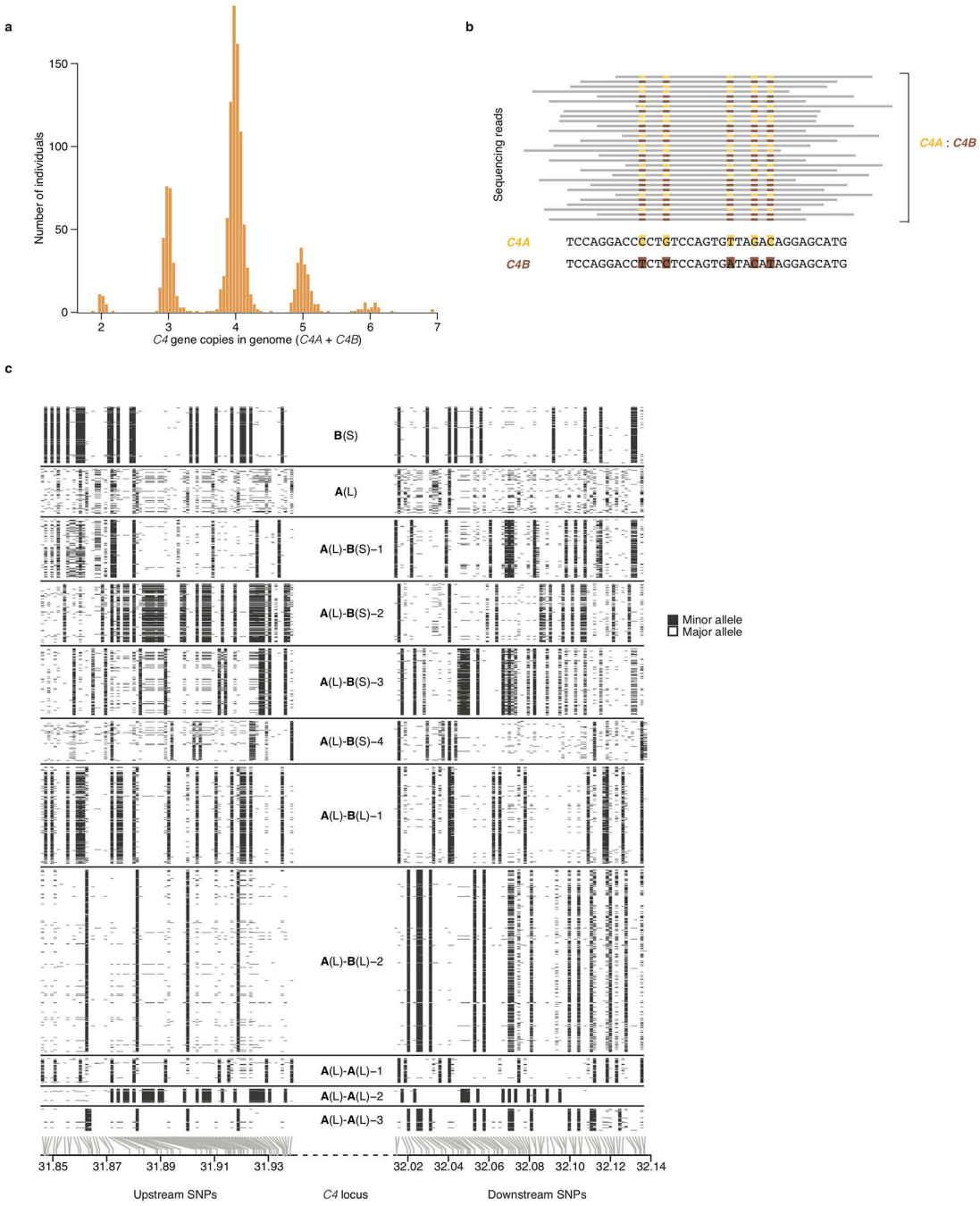
Blood plasma was collected and immunoturbidimetric measurements of C3 and C4 protein in 1,844 individuals (182 men, 1662 women) by Sjögren's International Collaborative Clinical Alliance (SICCA) from individuals with and without SjS as previously described[51]. *C4* copy numbers for these individuals were previously imputed for use in logistic regression of SjS risk. As *C4* copy number has an effect on measured C4 protein similar to CSF (Extended Data Fig. 7b), we normalized C4 levels to them in all following analyses. Estimation of average measurements by age for each sex was done by local polynomial regression smoothing (LOESS) on log-concentrations of C3 (mg/dL) and C4 (mg/[dL, per *C4* gene copy number]) protein (Extended Data Fig. 7c, d). To evaluate the significance of sex bias within age ranges displaying the greatest difference (informed by LOESS), we analyzed individuals in these bins with the non-parametric unsigned Mann-Whitney rank–sum test comparing concentration distributions for males and females.

Difference in C4 protein levels between individual with and without SjS was done by performing a non-parametric unsigned Mann-Whitney rank–sum test on C4 protein levels with and without normalization to *C4* genomic copy number (Extended Data Fig. 7e, f).

Data Availability Statement

Individual genotype data for Sjögren's syndrome cases and controls and individual plasma concentrations for C4 and C3 are available in dbGaP under accession number phs000672.v1.p1. Individual genotype data for schizophrenia cases and controls are available by application to the Psychiatric Genomics Consortium (PGC). Questions regarding individual genotype data for SLE cases and controls of European and/or African American ancestry can be directed to Timothy J. Vyse (timothy.vyse@kcl.ac.uk). Data resources (reference haplotypes), software scripts and instructions for imputing C4 alleles into SNP data sets are available on the McCarroll lab web site at http://mccarrolllab.org/resources/resources-for-c4/. Genotype and protein concentration data for CSF samples are available upon request.

Extended Data



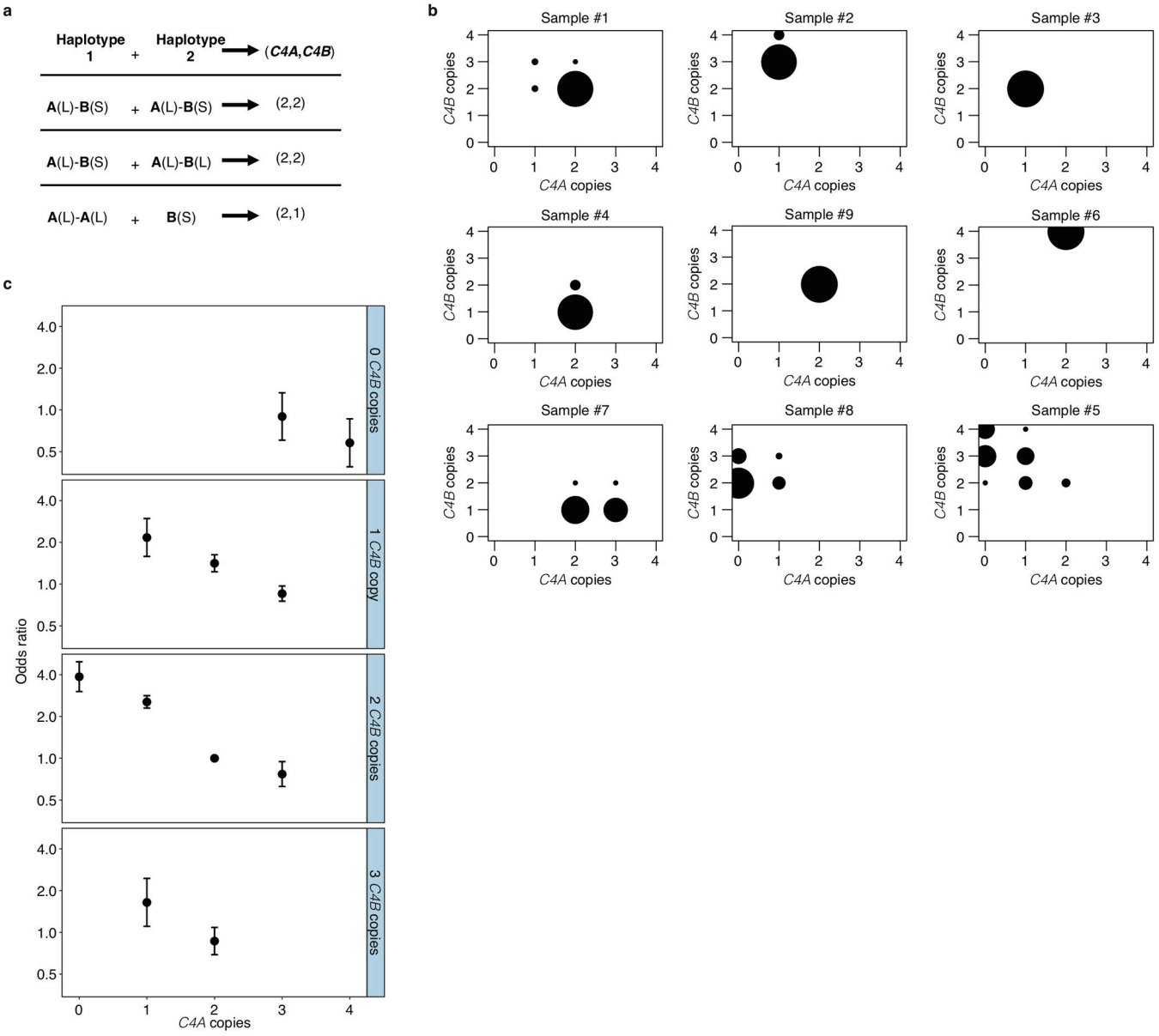
Extended Data Figure 1. A panel of 2,530 reference haplotypes (created from whole-genome sequence data) containing *C4* alleles and SNPs across the MHC genomic region enables imputation of *C4* alleles into large-scale SNP data.

(a) Distributions (across 1,265 individuals) of total *C4* gene copy number (*C4A* + *C4B*), as measured from read depth of coverage across the *C4* locus, in whole-genome sequencing data.

(b) The relative numbers of reads that overlap sequences specific to *C4A* or *C4B* (together with the total *C4* gene copy number as in **a**) are used to infer the underlying copy numbers of the *C4A* and *C4B* genes. For example, in an individual with four *C4* genes, the presence

of equal numbers of reads specific to *C4A* or *C4B* suggests the presence of two copies each of *C4A* and *C4B*. Precise statistical approaches (including inference of probabilistic dosages), and further approaches for phasing *C4* allelic states with nearby SNPs to create reference haplotypes, are described in Methods.

(c) The SNP haplotypes flanking each *C4* allele are shown as rows (SNPs as columns), with white and black representing the major and minor allele of each SNP. Gray lines at the bottom indicate the physical location of each SNP along chromosome 6. The differences among the haplotypes are most pronounced closest to *C4* (toward the center of the plot), as historical recombination events in the flanking megabases will have caused the haplotypes to be less consistently distinct at greater genomic distances from *C4*. The patterns indicate that many combinations of *C4A* and *C4B* gene copy numbers have arisen recurrently on more than one SNP haplotype, a relationship that can be used in association analyses (Fig. 1b).

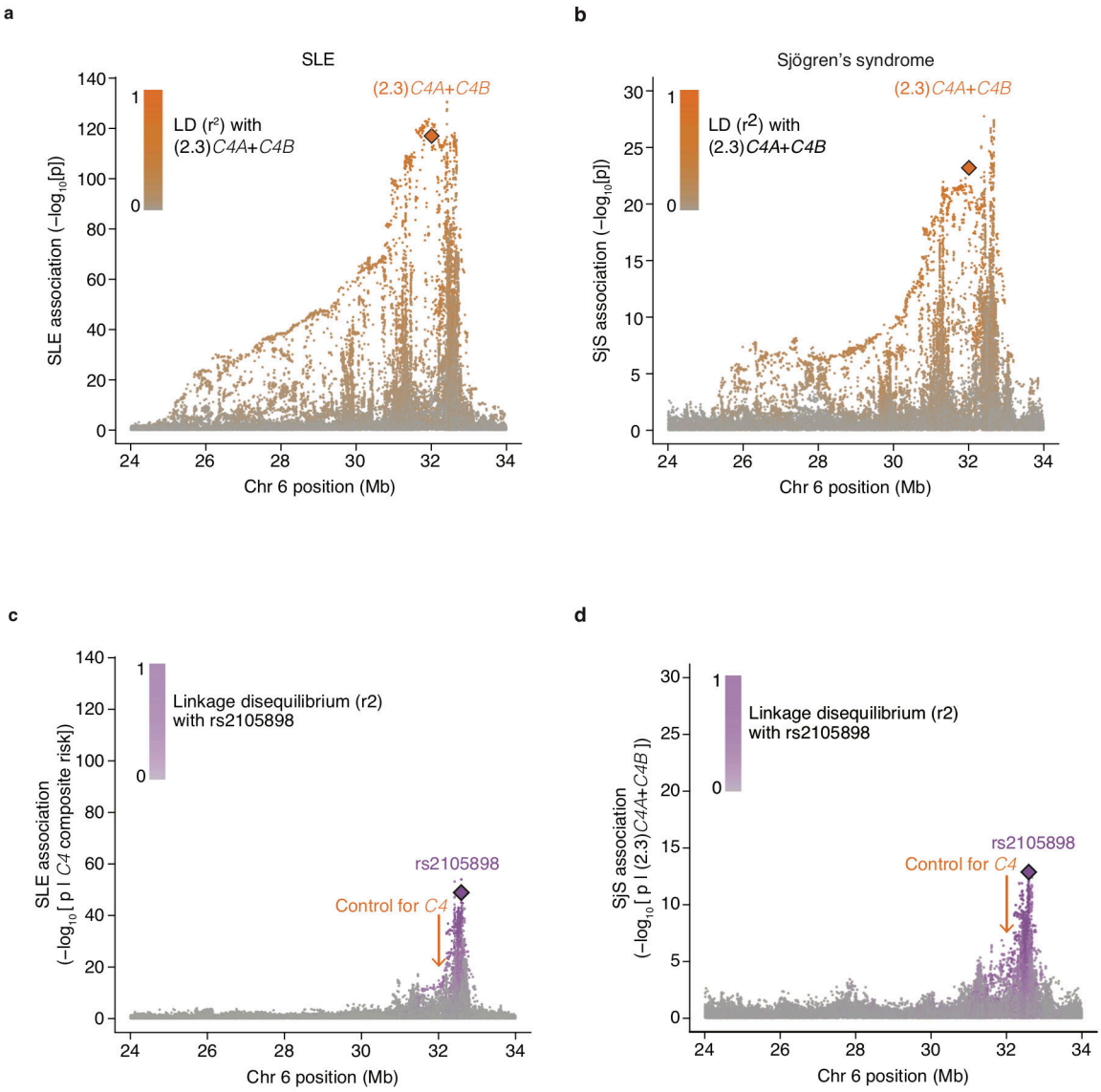


Extended Data Figure 2. Aggregation of joint *C4A* and *C4B* genotype probabilities per individual across imputed *C4* structural alleles for estimation of SLE risk for each combination.

(a) An individual's joint *C4A* and *C4B* gene copy number can be calculated by summing the *C4A* and *C4B* gene contents for each possible pair of two inherited alleles. Many pairings of possible inherited alleles result in the same joint *C4A* and *C4B* gene copy number.

(b) Each individual's *C4A* and *C4B* gene copy number was imputed from their SNP data, using the reference haplotypes summarized in Extended Data Fig. 1c. For >95% of individuals (exemplified by samples 1–6 in the figure), this inference can be made with >90% certainty/confidence (the areas of the circles represent the posterior probability distribution over possible *C4A/C4B* gene copy numbers). For the remaining individuals (exemplified by samples 7–9 in the figure), greater statistical uncertainty persists about *C4* genotype. To account for this uncertainty, in downstream association analysis, all *C4*

genotype assignments are handled as probabilistic gene dosages – analogous to the genotype dosages that are routinely used in large-scale genetic association studies that use imputation. (c) Odds ratios and 95% confidence intervals underlying each of the *C4*-genotype risk estimates in Fig. 1a presented as a series of panels for each observed copy number of *C4B*, with increasing copy number of *C4A* for that *C4B* dosage (x-axis). Data are from analysis of 6,748 SLE cases and 11,516 controls of European ancestry.



Extended Data Figure 3. Conditional association analyses for genetic markers across the extended MHC genomic region within the European-ancestry SLE and Sjögren's syndrome (SjS) cohort.

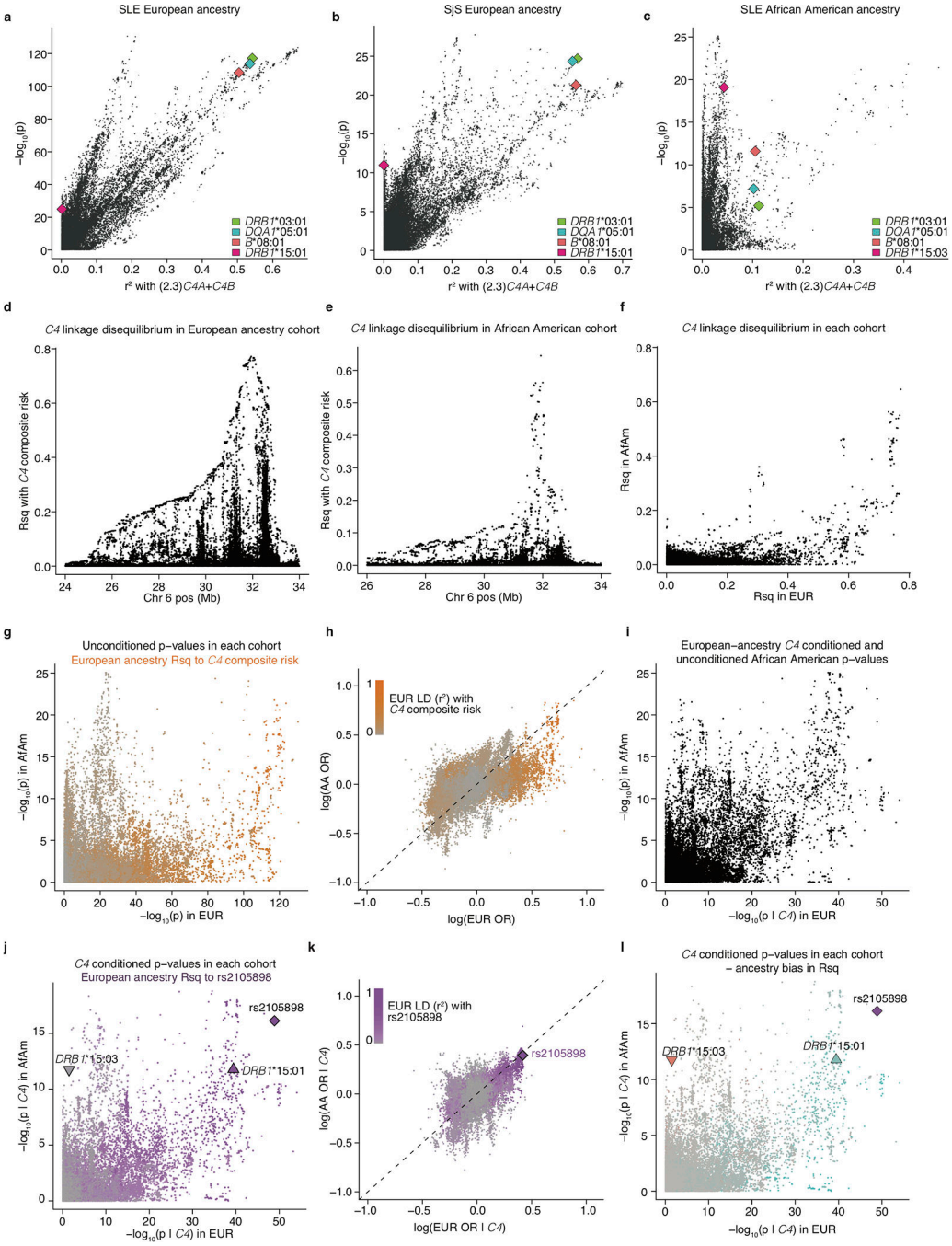
(a) Association of SLE with genetic markers (SNPs and imputed *HLA* alleles) across the extended MHC locus within the European-ancestry SLE cohort (6,748 cases and 11,516 controls). Orange diamond: an initial estimate of *C4*-related genetic risk, calculated as a weighted sum of the number of *C4A* and *C4B* gene copies: (2.3)*C4A*+*C4B*, with the weights derived from the relative coefficients estimated from logistic regression of SLE risk vs. *C4A* and *C4B* gene dosages. This risk score is imputed with an accuracy (r^2) of 0.77. Points representing all other genetic variants in the MHC locus are shaded orange according to their level of linkage disequilibrium–based correlation to this *C4*-derived risk score.

(b) As in **a**, but for a European-ancestry Sjögren's syndrome (SjS) cohort (673 cases and 1,153 controls). The orange diamond here also represents (2.3)*C4A*+*C4B*, with this

weighting derived from the relative coefficients estimated from logistic regression of SjS risk vs. *C4A* and *C4B* gene dosages

(c) Association of SLE with genetic markers (SNPs and imputed *HLA* alleles) across the extended MHC locus within the European-ancestry SLE cohort controlling for *C4* composite risk (weighted sum of risk associated with various combinations of *C4A* and *C4B*). Variants are shaded in purple by their LD with rs2105898, an independent association identified from trans-ancestral analyses.

(d) As in **c**, but in association with a European-ancestry SjS cohort. Here a simpler linear model of risk contributed by *C4A* and *C4B* was used instead of a weighted sum across all possible combinations.



Extended Data Figure 4. Using *C4* gene variation to understand the appearance of trans-ancestral disparity in MHC association signals, and to fine-map an additional genetic effect

All panels show association signals (for SLE and SjS) for variants in a multi-megabase region of human chromosome 6 containing the MHC region including the *HLA* and *C4* genes.

(a) Relationship between SLE association [-log$_{10}$(*p*), y-axis] and LD to the weighted *C4* risk score (x-axis) for genetic markers and imputed *HLA* alleles across the extended MHC locus. In this European-ancestry cohort, it is unclear (from this analysis alone) whether the association with the markers in the predominant ray of points (at a ~45° angle from the x-

axis) is driven by variation at *C4* or by the long haplotype containing *DRB1**03:01 (green), *DQA1**05:01 (blue), and *B**08:01 (red). In addition, at least one independent association signal (a ray of points at a higher angle in the plot, with strong association signals and only weak LD-based correlation to *C4* and *DRB1**0301) with some LD to *DRB1**15:01 (maroon) is also present.

(b) Analysis as in **a**, but for associations to SjS in a cohort of European ancestry. As in SLE, it is initially unclear whether the genetic association signal is driven by variation at *C4* or by linked *HLA* alleles, *DRB1**03:01 (green), *DQA1**05:01 (blue), and *B**08:01 (red). There is also an independent association signal with LD to *DRB1**15:01 (maroon).

(c) Analysis as in **a**, but of an African American SLE case–control cohort (in which LD in the MHC region is more limited). Many MHC-region SNPs associate with SLE in proportion to their LD with the weighted *C4* risk score inferred from the earlier analysis of the European-ancestry cohort; this *C4*-derived risk score itself associates with SLE at $p = 4.3\times10^{-19}$ in a logistic regression on 1,494 SLE cases and 5,908 controls. No similarly strong association is observed for *DRB1**03:01, *DQA1**05:01, or *B**08:01, *HLA* alleles which are in strong LD with *C4* risk on European-ancestry (but not African American) haplotypes. An independent association signal is also present in this cohort, more clearly in LD with the *DRB1**15:03 allele (maroon).

(d) LD in the European-ancestry SLE cohort between the composite *C4* risk term (weighted sum of risk associated with various combinations of *C4A* and *C4B* from Fig. 2a) and variants in the MHC region as r^2 (y-axis).

(e) As in **d**, but for the African American SLE cohort.

(f) LD (to *C4* composite risk) for the same variants in European-ancestry individuals (x-axis) and African Americans (y-axis). Note the abundance of variants that have greater LD with *C4* risk among European-ancestry individuals than among African Americans. Also, several groups of variants have equivalent LD (to *C4* risk) in European ancestry individuals but exhibit a range of LD to *C4* risk among African Americans.

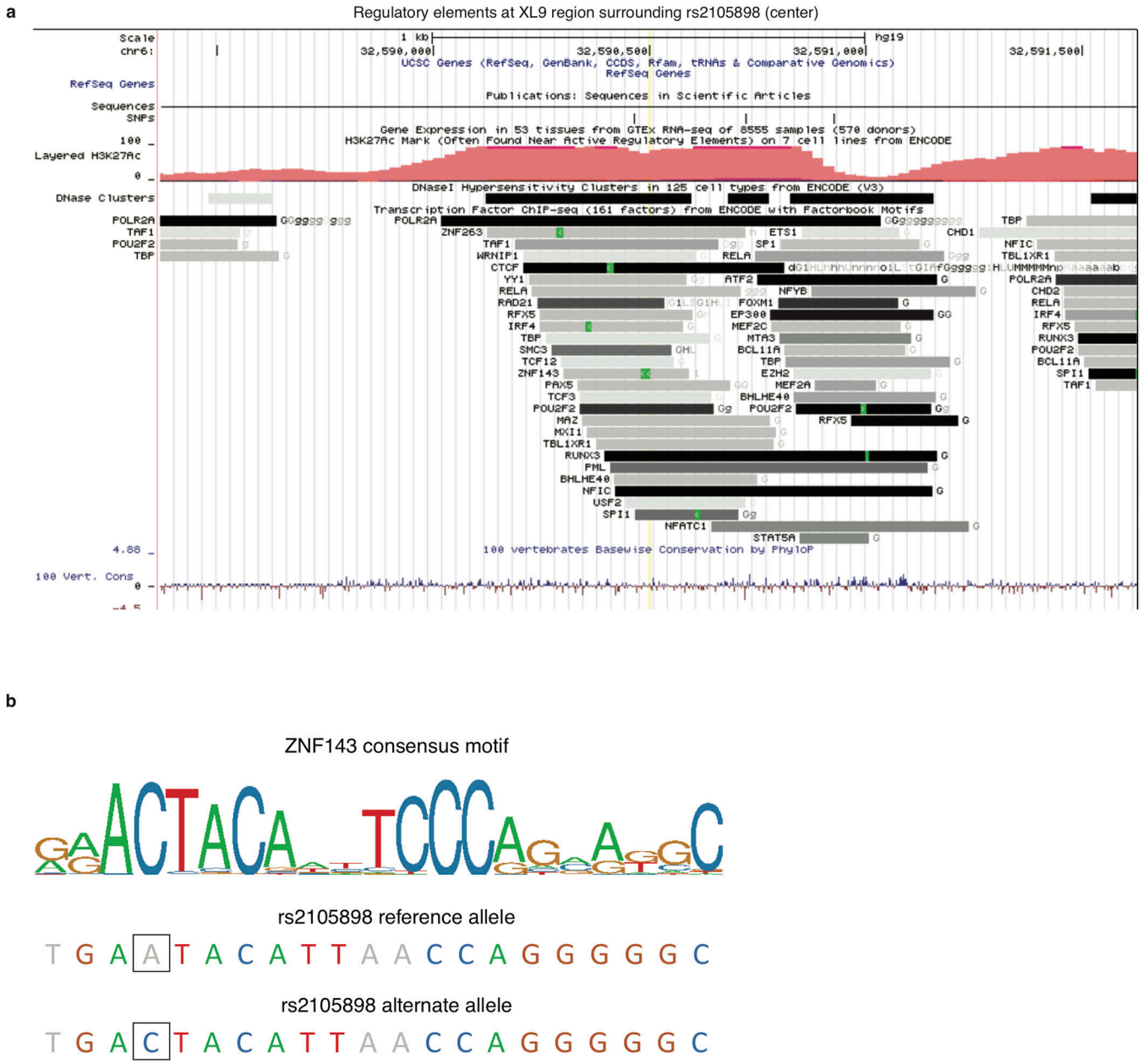
(g) Associations with SLE ($-\log_{10}$ p-values) for the same variants in European ancestry (x-axis) and African American (y-axis) case-control cohorts. Orange shading represents the extent of LD with *C4* risk in European ancestry individuals. Variants with strong European-specific association to SLE are generally in strong LD with *C4* risk among Europeans-ancestry individuals.

(h) Comparison of the inferred effect size from association of genetic markers with SLE (unconditioned log-odds ratios) among European-ancestry (x-axis) and African American (y-axis) research participants. As also seen in **g**, variants with discordant associations to SLE (across populations) tend also to be in strong LD to *C4* risk among European-ancestry individuals.

(i) As in **g**, but now controlling for the effect of *C4* variation in analysis of the European-ancestry cohort (x-axis). Note that controlling for *C4* risk in European-ancestry individuals alone greatly aligns (relative to **g**) the patterns of association between European ancestry and African American cohorts.

(j) As in **i**, but now also controlling for the effect of *C4* in associations of the African American cohort. Note that due to the lack of strong LD relationships between *C4* and variants in the MHC region in African Americans (**e**), this further adjustment does not change results strongly (relative to **i**). The independent signal, rs2105898, and *HLA* alleles,

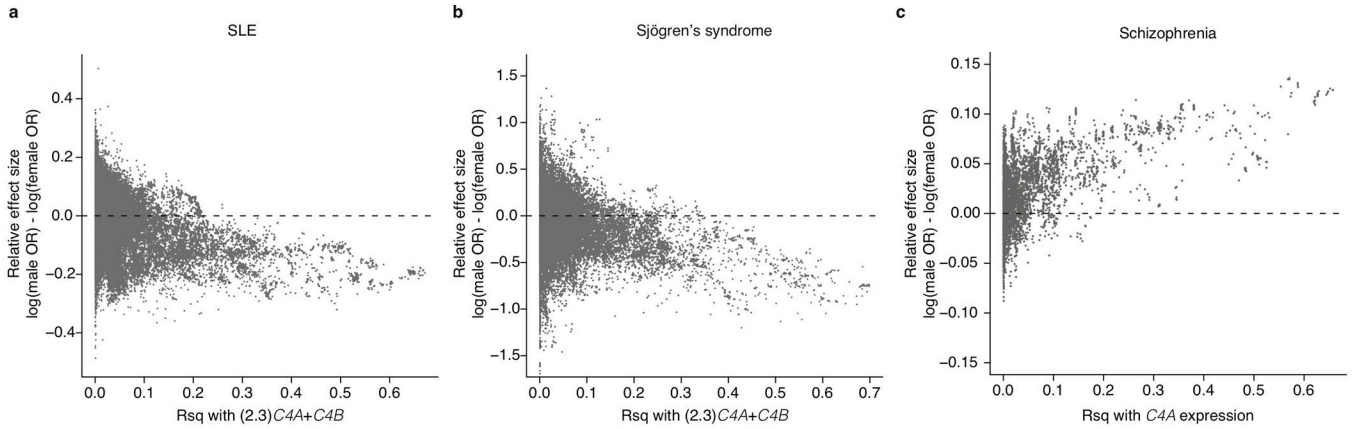
*DRB1**15:01 and *DRB1**15:03, are also highlighted. LD with rs2105898 in European-ancestry individuals is indicated by purple shading.
(k) Comparison of the inferred effect sizes from association of genetic markers with SLE (log-odds ratios) controlling for *C4*-derived risk among European-ancestry (x-axis) and African American (y-axis) research participants. Two SNPs (rs2105898 and rs9271513) that form a short haplotype common to both ancestry groups are among the strongest associations in both cohorts. (Their association to SLE in the European-ancestry cohort was initially much less remarkable than that of other SNPs that are in strong LD with *C4*.) LD with rs2105898 in European-ancestry individuals is indicated by purple shading.
(l) As in **i**, but with variants shaded by whether they exhibit greater LD to rs2105898 in Europeans (blue) or African Americans (red).



Extended Data Figure 5. Relationship of rs2105898 alleles to a known ZNF143 binding motif in the XL9 region of the MHC class II locus

(a) Location of rs2105898 (yellow line at center) within the XL9 region, with relevant tracks showing overlapping histone marks and transcription factor binding peaks (from ENCODE[52]), visualized with the UCSC genome browser[53].

(b) ZNF143 consensus binding motif as a sequence logo, with the letters colored if the base is present in >5% of observed instances. The alleles of rs2105898 are indicated by outlined box surrounding the base.

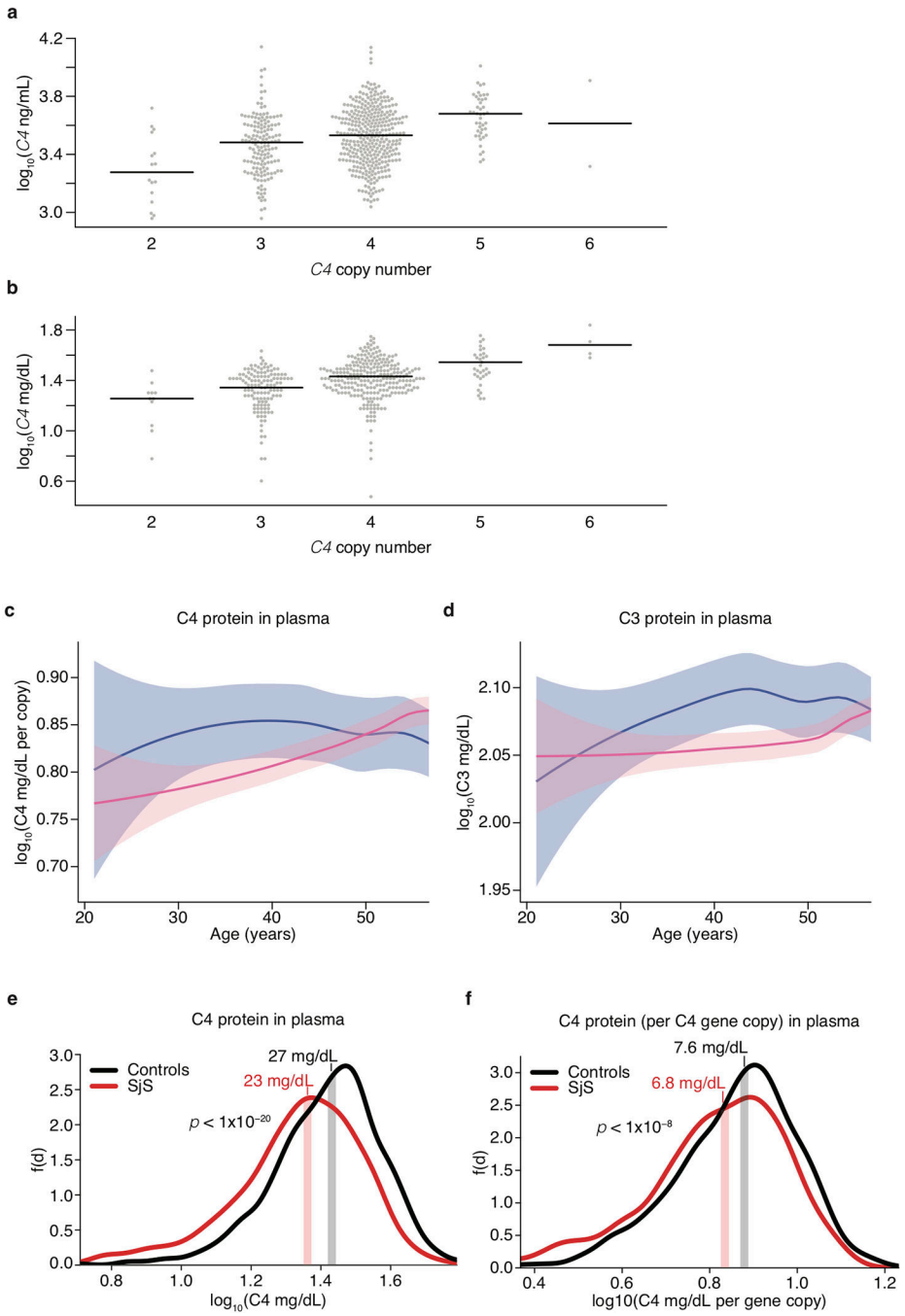


Extended Data Figure 6. Relationships between sex bias of disease associations and LD to *C4* risk for variants in the MHC region.

(e) Relationship between male bias in SLE risk (difference between male and female log–odds ratios) and LD with *C4* risk for common (minor allele frequency [MAF] > 0.1) genetic markers across the extended MHC region. For each SNP, the allele for which sex risk bias is plotted is the allele that is positively correlated (via LD) with *C4*-derived risk score.

(f) Relationship between male bias in SjS risk (log-odds ratios) and LD with *C4* risk for common (minor allele frequency [MAF] > 0.1) genetic markers across the extended MHC region. For each SNP, the allele for which sex risk bias is plotted is the allele that is positively correlated (via LD) with *C4*-derived risk score.

(g) Relationship of male bias in schizophrenia risk (log–odds ratios) and LD to *C4A* expression for common (MAF > 0.1) genetic markers across the extended MHC region. For each SNP, the allele for which sex risk bias is plotted is the allele that is positively correlated (via LD) with imputed *C4A* expression, as previously described[7].



Extended Data Figure 7. Correlation of C4 protein measurements in cerebrospinal fluid and blood plasma with imputed *C4* gene copy number and relationship of plasma complement to sex and SjS status

(a) Measurements of C4 protein in CSF obtained by ELISA are presented as $\log_{10}$(ng/mL) (y-axis) for each observed or imputed copy number of total *C4* (x-axis, here showing most likely copy number from imputation). Because *C4* gene copy number affects C4 protein levels so strongly, we normalized C4 protein measurements to each donor's *C4* gene copy number in subsequent analyses (Fig. 3c). Bars indicate median values for each *C4* copy number.

(b) Measurements of C4 protein in blood plasma obtained by immunoturbidimetric assays are presented as $\log_{10}$(mg/dL) (y-axis) for each imputed most-likely copy number of *C4* genes (x-axis). Because *C4* gene copy number affects C4 protein levels so strongly, we normalized C4 protein measurements by *C4* gene copy number in subsequent analyses as in **c**. Due to the number of observations (n = 1,844 total), the plot is downsampled to 500 points; the median bars shown are for all individuals (before downsampling).
(c) Levels of C4 protein in blood plasma from 182 adult men and 1662 adult women as a function of age. Concentrations are normalized to the number of *C4* gene copies in an individual's genome (a strong independent source of variance) and shown on a $\log_{10}$ scale as a LOESS curve. Shaded regions represent 95% confidence intervals derived during LOESS.
(d) Levels of C3 protein in blood plasma as a function of age from the same individuals in panel **c**. Concentrations are shown on a $\log_{10}$ scale as a LOESS curve. Shaded regions represent 95% confidence intervals derived during LOESS.
(e) C4 protein in blood plasma was measured in 670 individuals with SjS (red) and 1,151 individuals without SjS (black) and is shown on a $\log_{10}$ scale (x-axis). Vertical stripes represent median levels for cases and controls separately. Comparison of the two sets was done with a non-parametric two-sided Mann-Whitney rank–sum test ($p = 4.8\times10^{-21}$).
(f) As in **e**, but concentrations are normalized to the number of *C4* gene copies in an individual's genome and this per-copy amount is shown on a $\log_{10}$ scale (x-axis). Comparison of the two sets was done with a non-parametric two-sided Mann-Whitney rank–sum test ($p = 7.6\times10^{-9}$).

Extended Data Table 1.
Imputation accuracy for *C4* copy numbers in European ancestry and African American haplotypes.

Imputation accuracy was evaluated by correlation of imputation results to *C4* gene copy numbers directly inferred from WGS data. Aggregated copy numbers imputed from each round of leaving 10 individuals out were correlated with the directly-typed measurements and are reported as r^2 for each feature of *C4* structural variation for European ancestry and African American members of the reference panel separately.

	Imputation accuracy (r^2)	
Gene copy number	**European ancestry**	**African Americans**
C4	0.80	0.58
C4A	0.78	0.65
C4B	0.74	0.61
C4-HERV	0.91	0.76
2.3(*C4A*)+*C4B*	0.77	0.64

Extended Data Table 2.

Frequency of common *C4* alleles and their LD-based correlation with *HLA* alleles in European ancestry and African American cohorts.

For each common *C4* allele and *HLA* gene, the allele with strongest LD (r^2) is listed if present on more than half of the haplotypes with that *C4* allele (exact fraction in %). r^2 values greater than 0.4 are highlighted to point out particularly strong *C4-HLA* allele correlations, such as for several *HLA* alleles with the *C4*-B(S) allele in European ancestry individuals. Some common *C4* alleles are further subdivided into distinct haplotypes used in imputation (and in Fig. 1b), as defined by shared alleles from variants flanking *C4*. Note that some alleles such as *C4*-A(L)-A(L)-3 are present at a low frequency in African Americans that might reflect their presence on admixed European-origin haplotypes spanning this region, whereas others such as *C4*-B(S) are likely to also exist on African haplotypes – these differences between *C4* alleles are also reflected in the similarity of LD with *HLA* alleles to the corresponding row of the European ancestry section.

European ancestry

A			*B*			*C*			*C4* allele	Allele Frequency	*DRB1*			*DQA1*			*DQB1*	
allele	**%**	**r^2**	**allele**	**%**	**r^2**	**allele**	**%**	**r^2**			**allele**	**%**	**r^2**	**allele**	**%**	**r^2**	**allele**	**%**
01:01	69	0.27	08:01	93	**0.75**	07:01	93	**0.57**	B(S)	13.7%	03:01	94	**0.71**	05:01	94	**0.7**	02:01	94
									A(L)	4.8%								
						06:02	69	0.31	A(L)-B(S)-1	6.1%	07:01	74	0.25	02:01	74	0.25		
			44:03	54	0.28	16:01	53	0.39	A(L)-B(S)-2	4.5%	07:01	57	0.1	02:01	57	0.1	02:02	55
									A(L)-B(S)-3	3.8%								
									A(L)-B(S)-4	4.5%								
			07:02	64	**0.42**	07:02	63	0.35	A(L)-B(L)-1	15.5%	15:01	73	**0.49**	01:02	74	0.32	06:02	70
									A(L)-B(L)-2	23.1%								
			35:01	55	0.2	04:01	57	0.09	A(L)-A(L)-1	3.2%	01:01	65	0.14	01:01	65	0.11	05:01	64
									A(L)-A(L)-2	2.1%	13:01	67	0.16	01:03	65	0.13	06:03	67
02:01	65	0.03	44:02	74	0.24	05:01	72	0.23	A(L)-A(L)-3	4.5%	04:01	80	0.29	03:03	79	0.37	03:01	82

African American

A			*B*			*C*			*C4* allele	Allele Frequency	*DRB1*			*DQA1*			*DQB1*	
allele	**%**	**r^2**	**allele**	**%**	**r^2**	**allele**	**%**	**r^2**			**allele**	**%**	**r^2**	**allele**	**%**	**r^2**	**allele**	**%**
									B(S)	5.0%				01:02	51	0.01		
									A(L)	7.5%								

									A(L)-B(S)-1	14.1%									
									A(L)-B(S)-2	18.1%									
									A(L)-B(S)-3	17.7%									
									A(L)-B(S)-4	6.5%									
									A(L)-B(L)-1	4.4%	15:01	67	0.2	01:02	72	0.04	06:02	59
									A(L)-B(L)-2	4.5%									
									A(L)-A(L)-1	0.7%	01:01	57	0.07	01:01	53	0.01		
									A(L)-A(L)-2	0.8%									
02:01	72	0.03	44:02	86	0.31	05:01	78	0.17	A(L)-A(L)-3	0.8%	04:01	93	0.27	03:03	86	0.14	03:01	87	

Extended Data Table 3.

Results of association analyses of SLE risk against *C4* variation, *HLA* alleles, and/or rs2105898 in European ancestry and African American cohorts.

Coefficients (beta, standard error) and p-values (as $-\log_{10}(p)$) for individual terms composing several relevant logistic regression models for predicting SLE risk in a European ancestry cohort of 6,748 SLE cases and 11,516 controls and an African American cohort of 1,494 SLE cases and 5,908 controls. Each analysis also included ancestry-specific covariates. For each model, the Akaike information criterion (AIC) and overall p-value (as determined by Chi-squared likelihood-ratio test) are given at the right to indicate the relative strengths of similar models for each ancestry cohort.

European ancestry

	C4			*C4A*			*C4B*			*DRB1**03:01			*B**
Model	beta	se	−log10(p)	beta	se	−log10(p)	beta	se	−log10(p)	beta	se	−log10(p)	beta
C4	−0.55	0.027	92.7										
C4A				−0.53	0.024	105.3							
C4A+C4B				−0.62	0.028	112	−0.27	0.037	12.3				
*DRB1**03:01										0.7	0.03	117.1	
*B**08:01													0.69
rs2105898													
C4A + *C4B* + *DRB1**03:01				−0.35	0.041	17.2	−0.11	0.041	2.3	0.4	0.046	17.5	
C4A + *C4B* + *B**08:01				−0.41	0.039	24.6	−0.17	0.039	4.7				0.35

C4A + *C4B* + rs2105898				−0.67	0.028	122.8	−0.32	0.038	16.4					
African American														
	C4			*C4A*			*C4B*			*DRB1**03:01			*B*	
Model	beta	se	−log10(p)	beta	se	−log10(p)	beta	se	−log10(p)	beta	se	−log10(p)	beta	
C4	−0.51	0.059	17.3											
C4A				−0.43	0.062	11.2								
C4A+C4B				−0.62	0.068	18.7	−0.41	0.068	8.6					
*DRB1**03:01										0.41	0.091	5.2		
*B**08:01													0.78	0.11
rs2105898														
C4A + *C4B* + *DRB1**03:01				−0.59	0.073	15	−0.38	0.071	7.1	0.1	0.099	0.5		
C4A + *C4B* + *B**08:01				−0.51	0.073	11.7	−0.37	0.069	7.2				0.49	0.12
C4A + *C4B* + rs2105898				−0.52	0.07	13.2	−0.43	0.069	9.4					

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

This work was supported by the National Human Genome Research Institute (HG006855), the National Institute of Mental Health (MH112491, MH105641, MH105653), the Stanley Center for Psychiatric Research, and the National Institute for Health Research Biomedical Research Centre (NIHR BRC) at Guy's and St Thomas' NHS Foundation and King's College London. We thank Christina Usher and Christopher Patil for contributions to the figures and manuscript text. We thank Marta Florio for suggestions regarding figure display.

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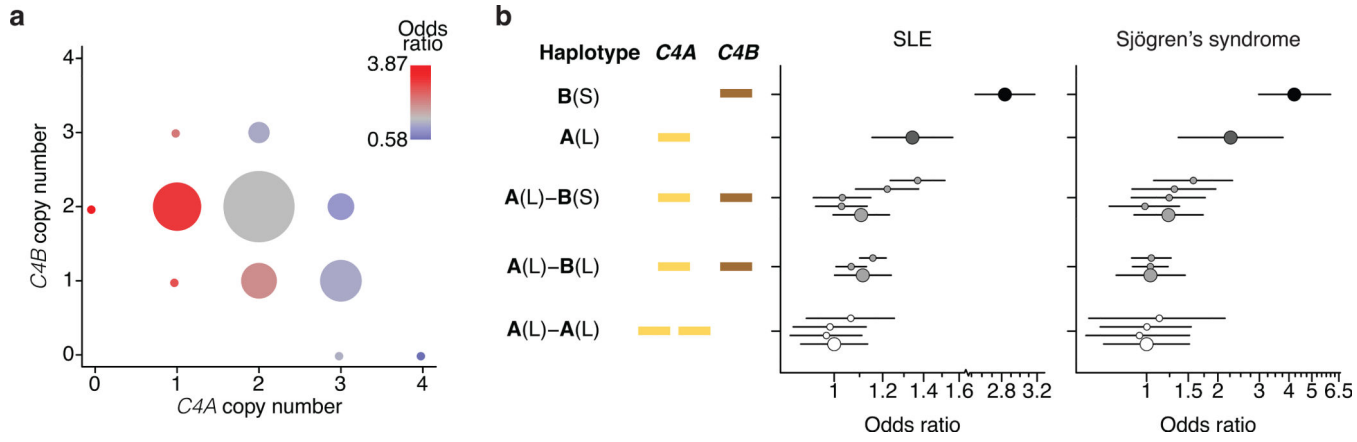

Figure 1. Association of SLE and Sjögren's syndrome (SjS) with *C4* alleles

(a) Levels of SLE risk associated with 11 common combinations of *C4A* and *C4B* gene copy number. The color of each circle reflects the level of SLE risk (odds ratio) associated with a specific combination of *C4A* and *C4B* gene copy numbers relative to the most common combination (two copies of *C4A* and two copies of *C4B*) in gray. The area of each circle is proportional to the number of individuals with that number of *C4A* and *C4B* genes. Paths from left to right on the plot reflect the effect of increasing *C4A* gene copy number (greatly reduced risk); paths from bottom to top reflect the effect of increasing *C4B* gene copy number (modestly reduced risk); and diagonal paths from upper left to lower right reflect the effect of exchanging *C4B* for *C4A* copies (modestly reduced risk). Data are from analysis of 6,748 SLE cases and 11,516 controls of European ancestry. The odds ratios are reported with confidence intervals in Extended Data Fig. 2c.

(b) SLE and SjS risk associated with common combinations of *C4* structural allele and MHC SNP haplotype. For each *C4* locus structure, separate odds ratios are reported for each "haplogroup," i.e., the MHC SNP haplotype background on which the *C4* structure segregates. Data are from analyses of 6,748 SLE cases and 11,516 controls for the left plot and 673 SjS cases and 1,153 controls for the right plot. Error bars represent 95% confidence intervals around the effect size estimate for each allele.

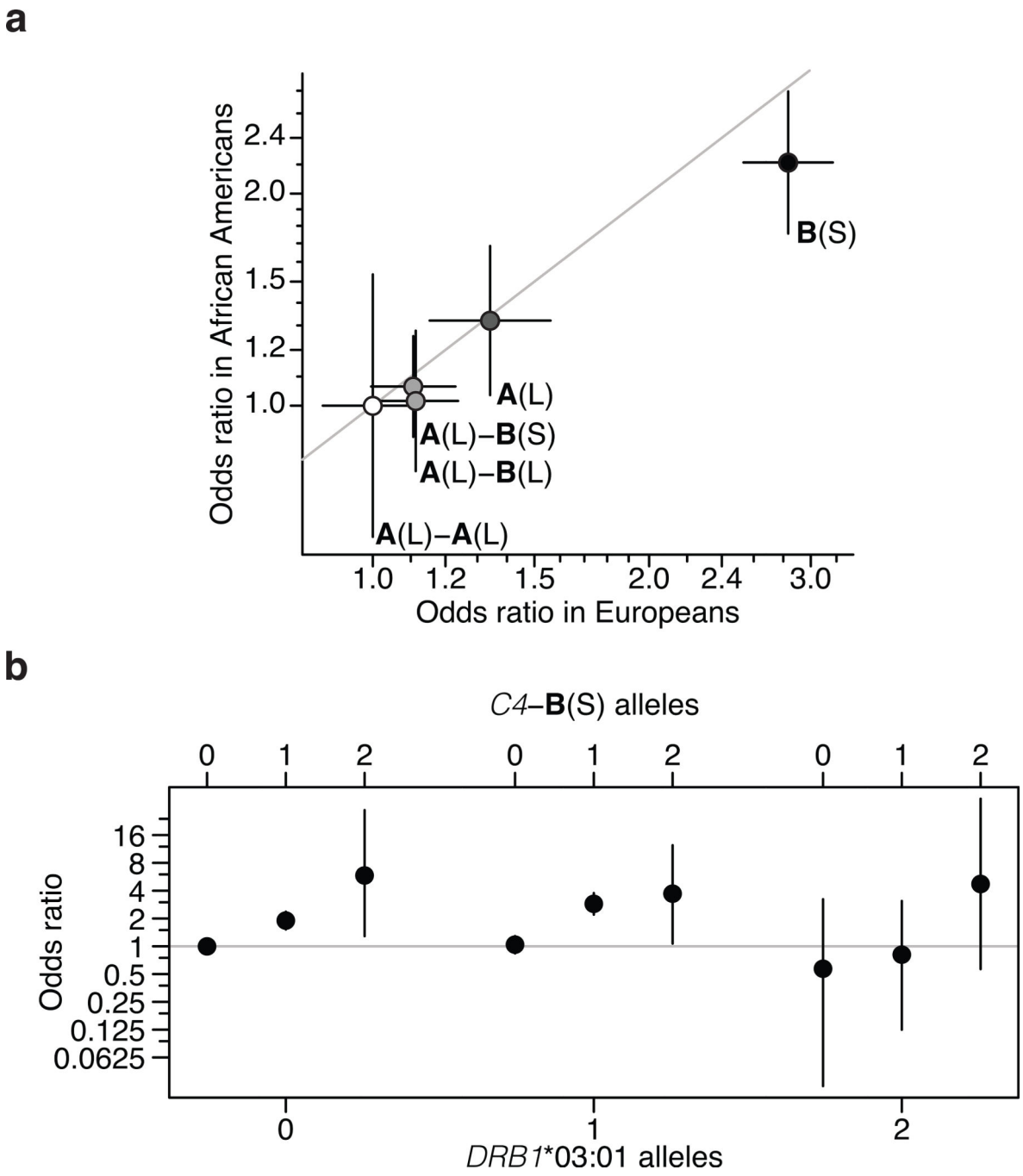

Figure 2. *C4* and trans-ancestral analysis of the MHC association signal in SLE

(a) Common *C4* alleles exhibit similar strengths of association (odds ratios) in European-ancestry and African American (1,494 SLE cases; 5,908 controls) cohorts. Error bars represent 95% confidence intervals around the effect size estimate for each sex.

(b) Analysis of SLE risk across combinations of *C4*-B(S) and *DRB1**03:01 genotypes in an African American SLE case–control cohort, in which the two alleles exhibit very little LD ($r^2 = 0.10$). On each *DRB1**03:01 genotype background, additional *C4*-B(S) alleles increase risk (i.e. within each grouping). Whereas on each *C4*-B(S) background, *DRB1**03:01 alleles

have no appreciable relationship with risk (this can be seen by comparing, for example, the first of the three points from each group). Error bars represent 95% confidence intervals around the effect size estimate for each combination of *C4*-B(S) and *DRB1**03:01.

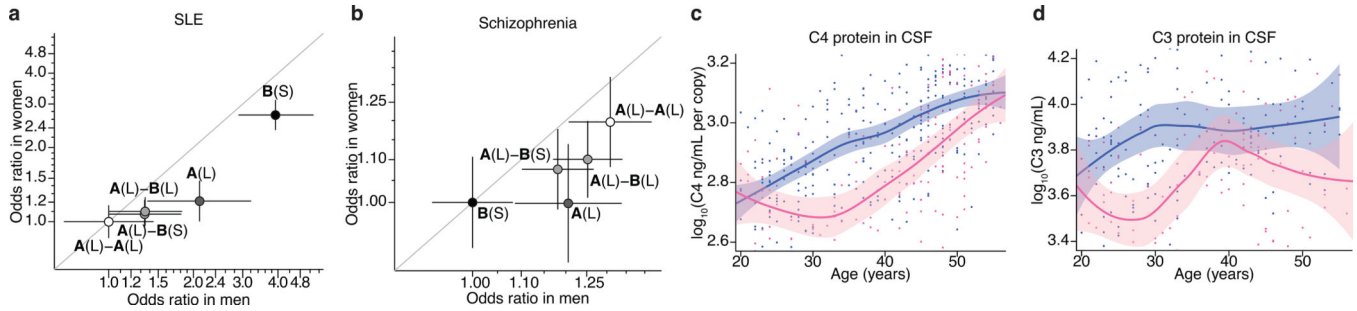

Figure 3. Sex differences in the magnitude of *C4* genetic effects and complement protein concentrations.

(a) SLE risk (odds ratios) associated with the four most common *C4* alleles in men (x-axis) and women (y-axis) among 6,748 affected and 11,516 unaffected individuals of European ancestry. For each sex, the lowest-risk allele (*C4*-A(L)-A(L)) is used as a reference (odds ratio of 1.0). Shading of each point reflects the relative level of SLE risk (darker = greater risk) conferred by *C4A* and *C4B* copy numbers as in Fig. 2b. Error bars represent 95% confidence intervals around the effect size estimate for each sex.

(b) Schizophrenia risk (odds ratios) associated with the four most common *C4* alleles in men (x-axis) and women (y-axis) among 28,799 affected and 35,986 unaffected individuals of European ancestry, aggregated by the Psychiatric Genomics Consortium[43]. For each sex, the lowest-risk allele (*C4*-B(S)) is used as a reference (odds ratio of 1.0). For visual comparison with **a,** shading of each allele reflects the relative level of SLE risk. Error bars represent 95% confidence intervals around the effect size estimate for each sex.

(c) Concentrations of C4 protein in cerebrospinal fluid sampled from 340 adult men (blue) and 167 adult women (pink) as a function of age with local polynomial regression (LOESS) smoothing. Concentrations are normalized to the number of *C4* gene copies in an individual's genome (a strong independent source of variance, Extended Data Fig. 7a) and shown on a $\log_{10}$ scale as a LOESS curve. Shaded regions represent 95% confidence intervals derived during LOESS.

(d) Levels of C3 protein in cerebrospinal fluid from 179 adult men and 125 adult women as a function of age. Concentrations are shown on a $\log_{10}$ scale as a LOESS curve. Shaded regions represent 95% confidence intervals derived during LOESS.