

Mapping of multiple susceptibility variants within the MHC region for 7 immune-mediated diseases

International MHC and Autoimmunity Genetics Network (IMAGEN)*

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The human MHC represents the strongest susceptibility locus for autoimmune diseases. However, the identification of the true predisposing gene(s) has been handicapped by the strong linkage disequilibrium across the region. Furthermore, most studies to date have been limited to the examination of a subset of the HLA and non-HLA genes with a marker density and sample size insufficient for mapping all independent association signals. We genotyped a panel of 1,472 SNPs to capture the common genomic variation across the 3.44 megabase (Mb) classic MHC region in 10,576 DNA samples derived from patients with systemic lupus erythematosus, Crohn's disease, ulcerative colitis, rheumatoid arthritis, myasthenia gravis, selective IgA deficiency, multiple sclerosis, and appropriate control samples. We identified the primary association signals for each disease and performed conditional regression to identify independent secondary signals. The data demonstrate that MHC associations with autoimmune diseases result from complex, multilocus effects that span the entire region.

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Following its discovery in mice in 1936 (1), the human MHC was mapped to the short arm of chromosome 6 and was studied extensively for both gene and variation content. The first full sequence of this region was completed and reported in 1999 by the MHC Sequencing Consortium (2). Gene density was greater than expected: from 224 identified loci, 128 were predicted to be expressed, and about 40% were predicted to have immunological functions. Among these loci, the classic HLA class I (*HLA-A*, *-B*, *-C*) and class II (*HLA-DP*, *-DQ*, *-DR*) gene clusters involved in antigen processing and presentation are the best characterized in terms of structure, diversity, and function.

The ability to respond to an antigen, whether foreign or self, and the nature of that response are determined to a large extent by the unique amino acid sequences of HLA alleles, an observation that followed the first association studies between HLA genotypes and susceptibility to diseases (3, 4). More than 100 diseases, many of which are autoimmune, have been associated with HLA genes. In some disorders, single HLA genes seem to be implicated in susceptibility (e.g., *HLA-B27* and ankylosing spondylitis) (5); in others, specific heterodimers (e.g., *DQB1*02* in celiac disease) (6) or complex interactions between alleles at multiple genes within the HLA (e.g., *HLA-DR*, *-DQ* and rheumatoid arthritis) have been described (7). In most cases, the MHC region is the strongest genetic component in autoimmune diseases (8–11). However, the extensive allelic variation and linkage disequilibrium (LD) across the MHC, together with the limited number of MHC genes examined in most association studies reported to date, have confounded attempts to resolve unequivocally the location of the primary signals responsible for disease susceptibility (12, 13).

To identify the genetic variants within the MHC that are disease specific and those that are shared across multiple inflammatory diseases, we examined a set of 10,576 DNA samples derived from patients with systemic lupus erythematosus (SLE), Crohn's disease (CD), ulcerative colitis (UC), rheumatoid arthritis (RA), myasthenia gravis (MG), selective IgA deficiency (IgAD), multiple sclerosis (MS), and appropriate control samples. Building on published high-density genetic maps of the

extended MHC (14–16), we genotyped a panel of 1,472 SNPs to capture the common genetic variation across the 3.44 megabase (Mb) classic MHC region. We imputed classic HLA alleles in individuals, which we validated in a subset of samples for which classic HLA typing was available. Although the majority of causal variants in this region remain to be identified, the results demonstrate that susceptibility to these diseases results from complex multilocus effects that span the entire region, with evidence for shared loci.

Results

SNP-Based Screening for Testing both HLA and non-HLA Variation.

Based on a high-resolution haplotype map of HLA alleles and SNPs across the extended MHC (16), we designed and genotyped a panel of 1,472 SNPs in a cohort of 10,576 individuals (supporting information (SI) Text), aiming to capture common variation across the MHC region including classic HLA alleles. The study participants were recruited according to well-established diagnostic and inclusion criteria (see *Materials and Methods*).

To maximize the uniformity of the genotype data and the comparability of results across all the cohorts, sample handling,

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Table 1. Top association SNP and HLA signals in screening and replication datasets

Disease	ID	Genomic position	Screen dataset				Replication dataset			
			Site	OR	P-value	Rank	Site	OR	P-value	Rank
SLE	rs1269852	32188168	UK	2.6	6.26E-19	1	US	2.00	6.23E-09	1
	DRB1*0301	32600001	UK	2.3	2.15E-15	8	US	1.87	8.78E-08	11
RA	rs6457617	32771828	SWE	0.3	3.1E-27	7	US	0.4	5.1E-16	2
	DQA1*0301	32716004	SWE	3.13	9.17E-19	1	US	4.8	1.91E-49	1
MS	rs3135391	32518964	UK	3.66	6.86E-29	2	US	3.2	7.03E-22	1
	DRB1*1501	32600020	UK	3.65	4.18E-29	1	US	3.1	1.22E-20	5

genotyping, allele calling, quality control, and association analyses were performed simultaneously on the entire set of DNA samples (see *SI Text*). To increase efficiency, 3 sets of shared controls were used, representing the general geographic regions from which the majority of patient samples were collected (the United States, the United Kingdom, and Sweden); this approach previously has been shown to be reliable (17). Overall, 83.8% of SNPs and 97.48% of all DNA samples passed quality-control thresholds (see *Methods*), with a final call rate of 99.0%.

We imputed the HLA alleles in all samples, using the high-resolution haplotype map of the region (including SNPs and HLA alleles) as the reference (16) (see *SI Text*). This study is the first to use imputation methodology for the association analysis of HLA alleles. Although imputation for SNP alleles now is well established, the high diversity and complex haplotype structure makes imputation of HLA alleles considerably more difficult (16, 18). Notably, the training data used here to make HLA allele predictions (16) is relatively small (range of 142–172 haplotypes with 4-digit resolution per locus), and low-frequency HLA alleles suffer from few observations in the training data (see ref. 16 for a list of classic alleles for which training data were available). We therefore measured the sensitivity, specificity, and positive predictive value of the imputed HLA alleles in a validation study using data available from the 1958 Birth Cohort (18) and data on samples from the current project for which classic HLA data

were available (Fig. S1 and Table S1). For the 16 alleles for which there is strong or suggestive evidence of disease association (as described in later sections), the average sensitivity is 92%, the average specificity is 98%, and the average positive predictive value is 86%. These figures indicate that, at least for alleles for which we make a claim of disease association, imputation accuracy is sufficient to expect little loss of power relative to direct typing.

We subsequently performed association tests for individual SNPs across the MHC region as well as for the (imputed) HLA alleles for each disease. For 3 of the diseases (SLE, RA, and MS), we exploited the availability of separate European and American cohorts, designating 1 cohort as the “screening cohort” and the other as the “replication cohort.” As shown in Table 1, the high degree of concordance for the top SNP and HLA alleles between cohorts indicates the robustness of the approach and the high quality of the sample collections. For example, the top 6 SLE markers (in both sides of the *RCCX* module and *HLA-DQA1*) are identical in both groups. Thus, for all subsequent analyses, samples were pooled to increase power. These composite results across all diseases are presented in Fig. S2, and the most highly associated SNPs and HLA alleles are summarized in Table 2. In fact, with the exception of MG, all the top associations observed in the separate cohorts are of genome-wide significance.

To determine whether the associations detected for the top

Table 2. Top disease specific association signals for the MHC in entire datasets

Disease	Type	ID	Genomic position	Top association signals relative position	OR	P-value	Reciprocal conditional p-value	r ² in disease dataset
SLE	SNP	rs1269852	32151660	Between TNXB and CREBL1	2.4	5.63E-29	1.64E-06	0.78
SLE	HLA	DRB1*0301	32605000	DRB1	2.1	1.06E-23	0.51	
UC	SNP	rs4639334	32653366	Between DRB1 and DQA1	4.0	1.30E-11	0.24	0.71
UC	HLA	DRB1*1101	32605000	DRB1	4.6	1.59E-11	0.32	
CD	SNP	rs382259	32280470	NOTCH4 region	2.3	1.40E-09	7.19E-06	0.14
CD	HLA	DRB1*1101	32605000	DRB1	2.2	2.08E-05	0.025	
RA	SNP	rs2395175	32513003	Between BTNL2 and DRA	3.7	1.36E-96	1.30E-05	0.78
RA	HLA	DQA1*0301	32660500	DQA1	4.0	1.78E-107	7.33E-15	
MG	SNP	rs2523674	31541152	3' of HCP5	1.5	2.89E-04	0.011	0.16
MG	HLA	HLA-C*0701	31346016	HLA-C	1.6	1.02E-03	0.047	
IgAD	SNP	rs2187668	32713861	Intronic DQA1	2.8	5.31E-14	0.69	0.66
IgAD	HLA	DQB1*0201	32676000	DQB1	2.8	3.04E-17	0.0013	
MS	SNP	rs3135391	32482210	DRA (synonymous coding)	3.4	5.51E-49	1	0.98
MS	HLA	DRB1*1501	32605000	DRB1	3.3	6.62E-48	1	

Table 3. Most significant secondary associations identified following conditioning on primary association signals

	Type	ID	Type	ID	Relative position	P-value
SLE	SNP	rs1269852	SNP	rs3135391	Within HLA-DRA	3.90E-06
			HLA	DRB1*1501		1.46E-05
UC	SNP	rs4639334	SNP	rs382259	NOTCH4 region	4.82E-06
			HLA	DQB1*0502		4.82E-03
CD	SNP	rs382259	SNP	rs4713436	Within CDSN	3.31E-04
			HLA	-	-	-
			HLA	rs6457614	Between HLA-DQA1 and HLA-DQA2	1.21E-17
RA	HLA	DQA1*0301	SNP	rs4713436		1.48E-21
			HLA	DQB1*0501		
MG	SNP	rs2523674	SNP	-	-	-
			HLA	-	-	-
IgAD	HLA	DQB1*0201	SNP	rs3135352	Between BTNL2 and HLA-DRA	7.70E-07
			HLA	DRB1*1501		6.65E-07
MS	SNP	rs3135391	SNP	rs2743951	Between HLA-F and HLA-G	1.88E-04
			HLA	HLA-B*4402		1.08E-08

Conditional logistic regression analysis was performed on each disease for the top HLA and top SNP. The top HLA and SNP signal following conditioning are shown for each of these alleles.

SNPs and the top HLA alleles are related or independent, we performed reciprocal logistic regression tests and also examined the correlation (as measured by the coefficient of determination, r^2) between these SNPs and the HLA alleles and generally found consistency between them (Table 2).

Next, we identified the set of variants that are statistically equivalent to each top (primary) association, providing a list of potential causal variants and the genomic region in which the causal gene is likely to be located (Table S2). Finally, we conditioned each disease dataset on the most significant association to identify secondary association signals independent of the peak association (Table 3). For many of the diseases, there was evidence for multiple additional association signals (Fig. 1 and Tables S3 and S4).

Systemic Lupus Erythematosus. In the combined United Kingdom and United States SLE dataset, the top signal is *rs1269852*,

located between *TNXB* and *CREBL1* (Table 2 and Fig. S2), in strong LD with *HLA-DRB1*0301* ($r^2 = 0.78$). Although the top imputed HLA allele is *HLA-DRB1*0301*, conditioning on the *TNXB-CREBL1* association indicates that the *HLA-DRB1*0301* signal may be dependent on this SNP, whereas the SNP itself shows association over and above that exhibited by *HLA-DRB1*0301* (Table 2). However, the imperfect correlation between the imputed and classically typed *HLA-DRB1*0301* allele in this dataset suggests that the impact of this class II association may be underestimated. Conditioning on the top signal (*rs1269852*) identifies a number of secondary signals; the best is *rs3135391* within *HLA-DRA* (Table 3) and variants in LD with this allele including *HLA-DRB1*1501* ($r^2 = 0.98$) (Table 3). Other signals potentially independent of *rs1269852* are seen in class I (between *RNF39* and *TRIM31*), class III (*NOTCH4*), and class II (*HLA-DQB1-DQA2*) (Fig. 1 and Table S4). The analysis detected further associations in class III; hence there seem to be at least 3 separate signals in this region tagged by the *SKIV2L* gene, *rs1269852*, and *NOTCH4* (19). Together, these data indicate the presence of multiple SLE risk alleles located across the class I, class II, and class III regions.

Ulcerative Colitis. Both the SNP and the HLA data convincingly show that the main signal (Table 2 and Fig. S2) is located in a narrow genomic window containing the *HLA-DRB1* gene (although < 10 kb from the 5' end of *HLA-DQA1*) and strongly suggest that the more common *HLA-DRB1*1101* allele ($\approx 10\%$) plays a primary role in UC susceptibility. An association with this allele also was detected by a recent meta-analysis of published data (4). This primary association to the *DRB1* locus is consistent with 3 recently published genome-wide association studies in UC (20–22). Interestingly, upon conditional analyses, the top secondary signal is localized around the *NOTCH4* gene (Table 3), a region also implicated in our analyses of SLE and CD. Independent of this signal is a cluster of associated alleles in the *BAT8-C2-RDBP-SKIV2L* region including intronic variants in each of the first 3 genes (Fig. 1 and Table S4). A role for this class III segment has not been characterized previously for UC but overlaps with 1 of the secondary signals in SLE, albeit to an independent set of alleles.

Crohn's Disease. Cross-disease risk factors in the MHC also seem to exist between CD and UC, because the current CD data reveal a significant association with the *HLA-DRB1*1101* allele (Table 2 and Fig. S2). Testing of the individual SNPs also provides evidence of association at *rs382259*, which is independent of

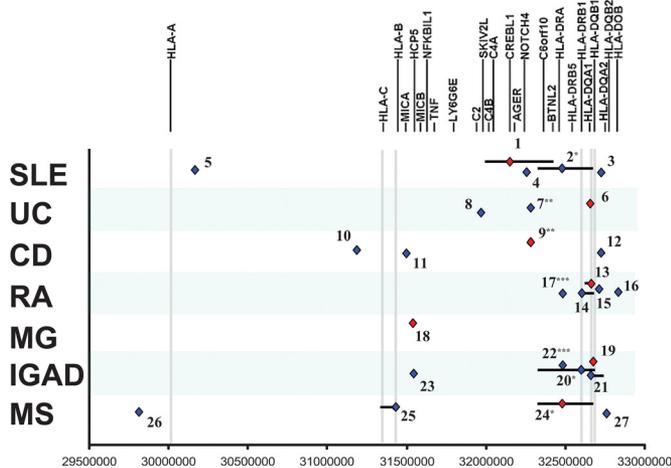


Fig. 1. Summary of primary and secondary signals for all 7 diseases. Top primary association signals (red) and putative independent secondary signals (blue) are shown for each disease along with their location within the region. Independent secondary signals were defined as those with residual conditional association p-values < 0.001, following logistic regression analysis for the top primary association signal and showing pair-wise correlation (r^2) lower than 0.2 with other neighboring signals. The correlation neighborhood or extent of correlation around each marker, at an $r^2 > 0.8$, is illustrated by black lines. Numbers attached to each signal refer to additional information on span and location of the correlation neighborhoods (see Table S4).

*HLA-DRB1*1101*, shows greater statistical significance, and is located in the adjacent *NOTCH4* region. Association with this SNP is supported by the significant association of an adjacent SNP (*rs419132*, $P = 1.0 \times 10^{-8}$), at a distance of less than 2 kb (pairwise r^2 between these 2 SNPs is 0.94). As noted earlier, this signal also is observed as the major secondary signal in UC. Furthermore, this same region was identified in the recent meta-analysis of CD genome-wide association studies (23), further supporting the importance of this region in CD risk. Searching the entire dataset for association signals independent of *rs382259*, we identified independent association signals in the class I region near the *CDSN* gene, in the region between *HLA-B* and *MICA*, and in the *DQA1-DQB1-DQA2-DQB2* region (Fig. 1, Table 3, and Table S4). Of interest, the *DQA1-DQB1-DQA2-DQB2* region contains 3 intronic SNPs in *HLA-DQA2* (*rs9276431*, *rs2213567*, and *rs2213568*) and a synonymous coding SNP in *HLA-DQB2* that are associated, albeit with p-values just shy of the significant threshold set for these conditional analyses. These results are consistent with previous reports of a modest association with *MICA* and *HLA-DRB1* alleles (*HLA-DRB1*0103*, *HLA-DRB1*04*, *HLA-DR7*, and *HLA-DRB3*0301*). These results also suggest an important role for the *HLA-DQA1/DQB* region, an observation that is consistent with a recent meta-analysis of CD genome-wide association studies (23). These results also suggest that previous reports of an association with TNF alleles (24) actually may represent a residual signal from the *MICA* association.

Rheumatoid Arthritis. Early studies in RA noted significant association with multiple alleles at the *HLA-DRB1* locus (**0101*, **0401*, and **0404*), and Gregersen et al. noted that this allelic heterogeneity could be explained by a shared amino acid sequence or “shared epitope” at positions 70–74 of the HLA-DRB1 protein (7). More recently, it has become evident that the RA association with the MHC is restricted to RA individuals positive for antibodies to citrullinated protein antigens (ACPA) (25). Therefore, the current study was restricted to the ACPA-positive form of RA. The top signal maps to *DQA1*0301*, and the top SNP is *rs2395175*, located ≈ 2.5 kb upstream from *HLA-DRA*. Although these 2 signals show high correlation ($r^2 = 0.78$), reciprocal conditional analysis reveals them to be independent (Fig. S2 and Table 2). *DQA1*0301* is reported to be in LD with *DRB1*0401* and **0404*, and although these shared-epitope alleles were not the top primary or secondary association signals, they were associated ($P = 4.07 \times 10^{-77}$ and 1.17×10^{-12} , respectively) and showed residual signal upon conditioning on *DQA1*0301* ($P = 0.0025$ and 2.28×10^{-5} , respectively).

Conditioning on either the top SNP or *DQA1*0301* identifies additional independent effects, the strongest of which is to *DQB1*0501* (Fig. 1 and Table 3). *DRB1*0101*, another shared-epitope allele, is part of this secondary signal, although with slightly lower (almost equivalent) association signal (see Table S4).

The data also suggest the presence of additional independent signals located within the class II region: *rs6457617* (located between *HLA-DQA1* and *-DQA2*), which was also independently reported by the Wellcome Trust Case Control Consortium study (17), *rs2621326* (located within the *HLA-DOB* locus) in a region previously reported by Lee and colleagues (26), and *rs3129878* (located in an intron of the *HLA-DRA* locus), a signal that also is shared with IGAD (see later sections). Additional significant signals can be observed beyond the ones described here, including the *HLA-DRB1*0404* allele (1 of the shared-epitope alleles) and a signal in the *HLA-DPB1-Col11A2* region, which have been reported previously (25, 26).

Myasthenia Gravis. In the MG dataset there is a paucity of significant signals in the class II region; the strongest associations arise from the class I region. Specifically, we observed that the

top association was to *rs2523674*, which is located 3.5 kb away from the 3' end of the HLA complex protein 5 (*HCP5*) gene, between *MICA* and *MICB*. The strongest imputed associated HLA allele is *HLA-C*0701*. There is strong LD between *HLA-C*0701* and *HLA-B8*, but the latter shows a slightly weaker association than *HLA-C*0701*. The SNP *rs2523674* and these HLA alleles are independent of each other (Table 2 and Fig. S2). The conditional analyses performed with *rs2523674* and *HLA-C*0701* did not reveal any statistically significant additional signals, although this result may be a reflection of the modest sample size and/or disease heterogeneity rather than a feature of the genetic architecture of this disease. Taken together, the data confirm the strong influence of *HLA-C* and suggest an independent effect of *rs2523674* in the *HCP5* region.

Selective IgA Deficiency. The association of IgAD with the MHC is clearly documented (27), but the precise location of the genetic effect within the region has remained elusive. The peak association in the current study is with the *HLA-DQB1*0201* allele ($P < 10^{-16}$) (Table 2 and Fig. S2). This association is $\approx 1,000$ -fold more significant than that of the imputed *HLA-DRB1*0301* and the top SNP (*rs2187668*) that tags the *DRB1*0301* allele. Conditioning upon *HLA-DQB1*0201* demonstrates that the *DRB1*0301* signal is not independent of the primary signal at *HLA-DQB1*. The analyses also reveal an independent secondary association with *HLA-DRB1*1501* that is protective (Table 3) as well as other suggestive risk alleles in the class II and class III regions located between *BTNL2* and *HLA-DRA* and in LD with the protective *DRB1*1501* allele (Fig. 1 and Table S4).

Multiple Sclerosis. The association of MS with HLA genes, specifically the *DRB1*1501* allele, has been a consistent finding across nearly all studies, including this study, in which the top HLA signal is *DRB1*1501*, detected both with a tagging SNP (*rs3135391*) and by imputed HLA (Table 2 and Fig. S2). Trends for association with *DRB1*0301* and *DRB1*0401* also were detected, with the *DRB1*0401* association nearly reaching a threshold for significance ($P < 10^{-5}$). Conditioning for *rs3135391*, putative independent clusters in the class III and class I regions emerge, with *HLA-B*4402* being the top signal (Fig. 1, Table 3, and Table S4). This allele is in LD with *HLA-C*0501* ($r^2 = 0.58$), an allele that has been associated with MS in the absence of *DRB1*1501* (28). When the MS cases were compared with unrelated controls, the results remained unchanged, and a case-control analysis demonstrated a similar under-representation of *HLA-B*4402* on *DRB1*1501* haplotypes from MS patients compared with controls. The significance of the residual signals in the *HLA-A* region after conditioning for *rs3135391* (Fig. S2 and Table S3) remains to be determined.

Discussion

In the multisystem autoimmune disease SLE, the MHC represents the strongest risk locus genomewide (10, 29, 30), and case-control studies in predominantly European-derived populations have revealed a consistent association with the *HLA-DRB1*0301* and *HLA-DRB1*1501* class II alleles and their respective haplotypes (31, 32). The particularly strong LD across these haplotypes has confounded attempts to identify clearly additional independent signals, such as those previously reported for complement C4-null alleles, as well as TNF and *SKIV2L* variants, all encoded within the class III region (19, 32, 33). The influence of copy number variation at the complement C4/RCCX locus in relation to the association signals demonstrated in this study remains to be established. The current high-density SNP analysis confirms that the predominant signals in SLE map to the class II and class III regions. Also, there seem to be at least 2 class III associations, with peaks on either side of the *RCCX* module, in addition to a further signal centered around

NOTCH4, a gene involved in development and cell fate; another independent signal is located in the class I region near *TRIM31*.

UC and CD are related inflammatory diseases of the gastrointestinal tract, classified together as inflammatory bowel diseases. Several independent genome-wide scans in inflammatory bowel disease have shown evidence of linkage to the MHC region (34–36), and recent genome-wide association studies of nonsynonymous SNPs in UC confirmed the association with the MHC, specifically within the *BTNL2* and *HLA-DRB1* genes (17, 37). In European-derived populations, *HLA-DRB1*0103* represents the most reproducible association observed to date in UC, albeit at a low prevalence of less than 2% (38). Previous studies of the HLA loci in CD have identified association with 4 independent HLA alleles, *DRB1*07*, *DRB1*0103*, *DRB1*04*, and *DRB3*0301*. One of 4 recent genome-wide association studies of CD, as well as the meta-analysis of these studies, also found association with this region, specifically with the nearby region delimited by the *HLA-DQA1* to *DQA2* genes (23, 39). The results from the current study, however, strongly implicate the more common *HLA-DRB1*1101* allele ($\approx 10\%$ allele frequency) in both UC and CD susceptibility. Upon conditional analyses, the top secondary signal is localized around *NOTCH4*, a gene also implicated in our analyses of SLE and CD.

In RA, the top independent signals map to *HLA-DQA1*0301* and *-DRB1*0101*, both shared-epitope alleles (7). Because different *DRB1* genotypes are known to modulate risk for RA in a hierarchical fashion, genotypic combinations for conditioning may be necessary to expose the full complexity of the underlying genetic association. For example, *DRB1*0401/0401* has been associated with very high risk, often in the range of an odds ratio between 20 and 30, whereas the combination of *DRB1*0101/*0401* is associated consistently with lower risk (40). Hierarchical risk profiles of different HLA alleles also are likely to be present in MS (41) and in other autoimmune disorders as well. Also, in RA, at least 2 other independent signals were observed, in the *DQ* region for *DQB1-0501* and in the *DPB1-CollIA2* region. These data thus indicate that multiple class II-related loci and alleles are associated only with ACPA-positive RA (25). This finding, in turn, may suggest that MHC class II alleles linked to this subset of RA may be involved in determining the magnitude of response to different citrullinated proteins/peptides.

MG is characterized by autoimmunity at the postsynaptic neuromuscular junction. Prior studies had suggested that the class I region has a role in susceptibility (42, 43). The current analysis reveals that, compared with the other autoimmune disorders, the genetic role of the MHC in MG is distinctive, consisting of a single signal in the class I region, specifically in the vicinity of the HLA complex protein 5 gene (*HCP5*) located between *MICA* and *MICB*. The strongest associated imputed HLA allele is *HLA-C*0701*. An underlying biologic heterogeneity of MG is likely; thus it will be of great interest to determine whether endophenotypes such as the identity of the autoantibody (acetylcholine receptor vs. muscle-specific kinase vs. titin), the presence of thymoma, or the occurrence of associated autoimmune diseases are associated with distinctive HLA-region signals. In this regard, an association with MHC class II genes has been reported in patients with muscle-specific kinase or titin antibodies (44).

Multiple HLA haplotypes are known to be positively associated with IgAD (serum IgA < 0.07 g/L), the most common form of primary immunodeficiency in the Western world (45, 46). The peak primary association in the current study is to the *HLA-DQB1*0201* allele, with a significant secondary association with *HLA-DRB1*1501* that is protective, and other putative risk alleles in the class II and class III regions. Recently, mutations in *MSH5*, encoded within the class III region, affecting class-switch recombination and associated with the *HLA-B14-DR1* haplotype, have been suggested to constitute a primary risk

factor for IgAD (47). However, no mutations in *MSH5* were identified on the *HLA-B8-DR3* susceptibility haplotype, suggesting a more complex MHC etiology in this disease.

Genetic studies of the HLA region in MS consistently yielded convincing evidence for the presence of a major susceptibility gene or genes (48). This signal maps primarily to the 1-Mb segment spanning the class II genes. There is debate, however, as to whether the *HLA-DRB1*1501* association explains the entire MHC genetic signal and whether other independent genes of interest exist within the class III, class I, and/or telomeric to the class I regions (28, 49). The results of the present study highlight the role of HLA class I gene products and are consistent with the known dose effect of *DRB1*1501* haplotypes on MS susceptibility suggesting that a second disease gene exists within the MHC (50) and also consistent with the current working model of MS pathogenesis which includes a prominent role for CD8+ T cells (51, 52). Further, MHC class I molecules may act as a molecular address for CD8+ T cells at the blood–brain barrier, facilitating the transendothelial migration of antigen-specific T-cells into the brain (53). This situation might have evolved as a result of selective pressure to promote antiviral immune surveillance of the central nervous system.

Because the conditional analysis used here is based generally on the assumption that a single primary association signal allows conditioning on a relatively homogeneous set of cases and controls that exhibit this primary association, additional layers of complexity are likely to exist. Nevertheless, the data clearly demonstrate that, in contrast to the prevailing single-locus model, the MHC associations with chronic inflammatory diseases result from complex, multilocus effects that span the entire MHC region. Furthermore, because this MHC-specific panel of SNPs was typed and analyzed uniformly across multiple diseases, it was possible to begin to identify a set of shared genetic variants (Fig. 1) that should, with additional mapping efforts, lead to a better understanding of common pathogenic mechanisms. For example, consistent independent signals across class I genes may indicate a possible role for killer cell immunoglobulin-like receptor-HLA class I combinations, perhaps by promoting innate immune responses to viral infections. In addition, although we highlight associations to specific HLA or non-HLA variants for each disease, we have shown that for any association signal there are many equivalent genetic variants, demonstrating that narrowing the associations and identification of the causal genes will require even larger disease cohorts, potentially in different populations of distinct ancestry. Finally, no single approach will be sufficient to dissect fully the complex set of HLA and non-HLA genetic factors, and thus a combination of the approach described herein with classic typing and deep resequencing will reveal the disease-specific and shared risk factors involved in chronic immune-mediated diseases.

Materials and Methods

Cohorts. The complete data set studied consisted of 10,576 individuals (*SI Text*). Diagnostic criteria, ascertainment protocols, and clinical and demographic characteristics for cases and controls are summarized in previously published reports: SLE (30), CD (54), UC (54), RA (55, 56), MG (57), IGAD (58), MS (48), and the 1958 birth cohort (59). Appropriate institutional review boards approved all studies, and written informed consent was obtained from all participants.

Genotyping Assay Design. We designed an Illumina GoldenGate panel of 1,536 SNPs consisting of 16 fingerprinting quality-control SNPs, 48 genomic control SNPs (60), 135 SNPs to tag the classic HLA types, and 1,337 SNPs to tag common SNP variations within the 3.44-Mb classic MHC region based on a high-density haplotype map using the Tagger algorithm (16, 61). All coordinates for SNPs and HLA markers are given relative to the National Center for Biotechnology Information Build 34 human genome assembly. Overall this set of SNPs captured variation of common ($\geq 5\%$) HLA markers, less-common ($< 5\%$) HLA markers, common non-HLA markers, and less-common non-HLA markers, with

an average maximum r^2 of 0.80, 0.64, 0.90, and 0.62, respectively. We imputed classic HLA alleles in all individuals based on the available SNP data (18).

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