Identification of Two Independent Risk Factors for Lupus within the MHC in United Kingdom Families

The Harvard community has made this article openly available. Please share how this access benefits you. Your story matters

Citation

Published Version
doi:10.1371/journal.pgen.0030192

Citable link
http://nrs.harvard.edu/urn-3:HUL.InstRepos:4454176

Terms of Use
This article was downloaded from Harvard University’s DASH repository, and is made available under the terms and conditions applicable to Other Posted Material, as set forth at http://nrs.harvard.edu/urn-3:HUL.InstRepos:dash.current.terms-of-use#LAA
Identification of Two Independent Risk Factors for Lupus within the MHC in United Kingdom Families

Michelle M. A. Fernando¹, Christine R. Stevens², Pardis C. Sabeti², Emily C. Walsh², Alasdair J. M. McWhinnie³, Anila Shah³, Todd Green², John D. Rioux²,4*, Timothy J. Vyse¹*

1 Section of Molecular Genetics and Rheumatology, Imperial College London, London, United Kingdom, 2 The Broad Institute of MIT and Harvard, Cambridge, Massachusetts, United States of America, 3 Histocompatibility Laboratories and Research Institute, The Anthony Nolan Trust, London, United Kingdom, 4 Université de Montréal, Montréal Heart Institute, Montréal, Québec, Canada

The association of the major histocompatibility complex (MHC) with SLE is well established yet the causal variants arising from this region remain to be identified, particularly due to inadequate study design and the strong linkage disequilibrium demonstrated by genes across this locus. The majority of studies thus far have identified strong association with classical class II alleles, in particular haplotypes [4], also known as ancestral haplotypes [5], sometimes spanning more than 2 Mb [6]. Thus, for many MHC-associated diseases, it has only been possible to delimit association signals to a particular extended haplotype or segment of one.

Introduction

Since the early 1970s, the human major histocompatibility complex (MHC) has been shown to be associated with a number of autoimmune, inflammatory, and infectious diseases, and it continues to be the focus of intense research [1]. The recently defined extended MHC (xMHC) encompasses 7.6 Mb of genome on 6p21.3 and is divided into five subregions from telomere to centromere: extended class I, classical class I, classical class III, classical class II, and extended class II. In addition, the MHC contains two hypervariable regions, the RCCX module in class III (spanning complement C4) and the HLA-DRB genes in class II, that both exhibit copy number polymorphism. Examination of the sequence across the extended MHC reveals the presence of 421 genes, and over 252 (60%) are thought to be expressed [2]. Around 40% of genes expressed within the classical MHC encode proteins with putative immunomodulatory function [3]. The classical class I and class II loci encode the human leucocyte antigen (HLA) proteins involved in antigen presentation to T cells, initiating the adaptive immune response. The class III region contains the greatest density of genes in the genome (58 expressed genes), which are often found in functionally related clusters [2].

A major obstacle in the identification of disease-specific causal variants within the MHC has been the strong linkage disequilibrium (LD) exhibited by certain alleles in this region, resulting in the existence of long-range, conserved, extended haplotypes [4], sometimes spanning more than 2 Mb [6]. Thus, for many MHC-associated diseases, it has only been possible to delimit association signals to a particular extended haplotype or segment of one.
Author Summary

Systemic lupus erythematosus (SLE/lupus) is a complex autoimmune disease in which the body’s immune system attacks its own tissues, causing inflammation in a variety of different organs such as the skin, joints, and kidneys. The cause of lupus is not known, but genes play a significant role in the predisposition to disease. The major histocompatibility complex (MHC) on Chromosome 6 contains at least 100 different genes that affect the immune system, including the genes with the strongest effect on lupus susceptibility. Despite the importance of the MHC in SLE, the identity of the actual genes in the MHC region that cause SLE has remained elusive. In the present study, we used the latest set of genetic markers present at the MHC in lupus families to identify the actual genes that affect the disease. To our knowledge, we have shown for the first time that two separate groups of genes are involved in SLE. One group of genes alters how the immune system may inappropriately target its own tissues in the disease. How the second set of genes predisposes to SLE is the subject of ongoing study.

In 2002, a family-based study employing microsatellites as surrogate markers for HLA-DRB1 haplotypes in Caucasian lupus families demonstrated association with DR3-, DR2- and DR8 (DRB1*0801)-containing haplotypes. In that study, Graham and colleagues reported that, taking advantage of recombinant chromosomes, the disease risk region could be limited to a 1 Mb region encompassing classical class II and class III [25]. We have performed a medium resolution association mapping study of the MHC in lupus families, utilizing a combination of SNPs and four-digit typing at the HLA-DRB1 locus in order to anchor haplotypes. Sixty-eight SNPs were successfully genotyped across a 2.4 Mb region of the MHC, from the class I locus KIAA1949 to the class II gene HLA-DPB2, in 314 UK Caucasian SLE trios. We used these data to perform a family-based association study in an attempt to distinguish the relative effects of the class II and class III regions of the MHC in lupus susceptibility. In addition, we employed the long-range haplotype test to search for the presence of high-frequency, extended haplotypes indicative of recent positive selection [26]. We have also used family-based and case-control strategies to examine genotypic risk at HLA-DRB1 and rs419788.

Results

Association Testing of HLA-DRB1 and MHC Region SNPs

In order to define the causal variation within the MHC region, we typed 314 complete SLE trios for the HLA-DRB1 gene as well as for 86 SNPs across a 2.4 Mb region encompassing the HLA class I locus HLA-B to HLA-DPB2. High-quality genotype data was obtained for HLA-DRB1 and 68 MHC SNPs (see Table S1 for quality control data). Association testing of the HLA-DRB1 gene revealed a significant association with HLA-DRB1*0301 (nominal \( p = 4.9 \times 10^{-8} \), permuted \( p < 0.0001 \), T:U = 129:55) in our lupus cohort (Table 1). There was also a trend for under transmission of the HLA-DRB1*0701 allele (nominal \( p = 0.0013 \), T:U 42:77); however, this association was no longer significant after correction for multiple testing as determined by 10,000 permutations of the dataset (permuted \( p = 0.09 \)). Furthermore, we did not find evidence of association with HLA-DRB1*1501 (nominal \( p = 1.0 \), T:U 70:70) or HLA-DRB1*0801 (nominal \( p = 1.0 \), T:U 11:11) in our cohort (see Table S2 for complete HLA-DRB1 association data); alleles previously suggested by microsatellite typing of a US lupus cohort [25].
Association testing of the MHC region SNPs also identified significant evidence of association to SLE (Table 1 for associated markers and Table S3 for all MHC SNPs). The SNP with the most significant association, rs419788 (nominal \( p = 4.3 \times 10^{-8} \), permuted \( p < 0.0001 \)) was of similar strength to that of the HLA-DRB1*0301 allele, with odds ratios (ORs) and 95% confidence intervals (CIs) of 2.0 (1.5–2.7) and 2.3 (1.7–3.3), respectively. This SNP is located within intron 6 of the TNF promoter, and suggested that there are two major independent association signals in the MHC in UK SLE: HLA-DRB1 and rs419788. The independence of the association signals at HLA-DRB1 and rs419788 is further supported by the observation that there is only modest LD between these two (\( r^2 = 0.24 \)). There was no association with any other HLA-DRB1 allele and the four SNPs independent of HLA-DRB1*0301 (TRANSMIT, unpublished data).

The association of the tumour necrosis factor gene promoter SNP TNF-308G/A with SLE is lost after conditioning for HLA-DRB1*0301 in our cohort. If we perform the reverse analysis and condition HLA-DRB1*0301 on the presence of the TNF promoter SNP, we find that the association remains, confirming that our TNF association is secondary to that of HLA-DRB1*0301.

Genotypic Risk for Class II and Class III Association Signals

Having established independent association at the allelic level with HLA-DRB1*0301 and rs419788 in our UK SLE cohort, we wanted to further determine the genotypic risk conferred by these variants and hence gain insight into their underlying mode of inheritance in lupus. We used case-control and family-based analyses to assess genotypic risk at HLA-DRB1, while the family-based test alone was used for

**Table 1. Single Marker Association and Conditional Regression Analysis in 314 UK SLE Trios**

<table>
<thead>
<tr>
<th>Marker #</th>
<th>Marker</th>
<th>Location</th>
<th>MAF</th>
<th>Minor Allele</th>
<th>Associated Allele</th>
<th>Single Marker Association</th>
<th>Conditional Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>rs2523589</td>
<td>Intergenic HLA-B and MICA</td>
<td>0.46</td>
<td>T</td>
<td>G</td>
<td>( 179:107, 2.1 \times 10^{-5} )</td>
<td>rs419788</td>
</tr>
<tr>
<td>4</td>
<td>rs2180629</td>
<td>TNF promoter</td>
<td>0.25</td>
<td>T</td>
<td>T</td>
<td>( 139:85, 3.0 \times 10^{-4} )</td>
<td>DRB1*0301</td>
</tr>
<tr>
<td>5</td>
<td>rs1052486</td>
<td>BAT3 exon 14</td>
<td>0.41</td>
<td>G</td>
<td>A</td>
<td>( 139:92, 2.4 \times 10^{-8} )</td>
<td>rs419788</td>
</tr>
<tr>
<td>6</td>
<td>rs605203</td>
<td>5′ SLC44A4</td>
<td>0.39</td>
<td>T</td>
<td>T</td>
<td>( 163:95, 2.3 \times 10^{-5} )</td>
<td>DRB1*0301</td>
</tr>
<tr>
<td>7</td>
<td>rs589428</td>
<td>EMHT2 intron 27</td>
<td>0.39</td>
<td>T</td>
<td>T</td>
<td>( 172:107, 1.0 \times 10^{-4} )</td>
<td>rs419788</td>
</tr>
<tr>
<td>8</td>
<td>rs419788</td>
<td>SKV2L intron 6</td>
<td>0.37</td>
<td>T</td>
<td>T</td>
<td>( 180:90, 4.3 \times 10^{-8} )</td>
<td>DRB1*0301</td>
</tr>
<tr>
<td>9</td>
<td>rs433061</td>
<td>TNXB intron 31</td>
<td>0.18</td>
<td>A</td>
<td>A</td>
<td>( 110:47, 5.0 \times 10^{-7} )</td>
<td>rs419788</td>
</tr>
<tr>
<td>10</td>
<td>rs204989</td>
<td>GPSM3 intron 3</td>
<td>0.26</td>
<td>A</td>
<td>A</td>
<td>( 140:78, 2.7 \times 10^{-5} )</td>
<td>DRB1*0301</td>
</tr>
<tr>
<td>11</td>
<td>rs3131296</td>
<td>NOTCH4 intron 18</td>
<td>0.21</td>
<td>T</td>
<td>T</td>
<td>( 150:73, 2.5 \times 10^{-7} )</td>
<td>rs419788</td>
</tr>
<tr>
<td>12</td>
<td>rs2227139</td>
<td>Intergenic HLA-DRD and HLA-DRB9</td>
<td>0.46</td>
<td>G</td>
<td>G</td>
<td>( 145:91, 4.0 \times 10^{-4} )</td>
<td>DRB1*0301</td>
</tr>
<tr>
<td>13</td>
<td>rs3219763</td>
<td>HLA-DRB1</td>
<td>0.21</td>
<td>A</td>
<td>A</td>
<td>( 129:55, 4.9 \times 10^{-8} )</td>
<td>rs419788</td>
</tr>
<tr>
<td>14</td>
<td>rs2040406</td>
<td>Intergenic HLA-DRB1 and HLA-DQA1</td>
<td>0.28</td>
<td>T</td>
<td>T</td>
<td>( 145:77, 5.0 \times 10^{-5} )</td>
<td>DRB1*0301</td>
</tr>
<tr>
<td>15</td>
<td>rs2187668</td>
<td>HLA-DQA1 intron 1</td>
<td>0.21</td>
<td>T</td>
<td>T</td>
<td>( 132:65, 1.8 \times 10^{-6} )</td>
<td>rs419788</td>
</tr>
</tbody>
</table>

a: 10,000 permutations in Haploview.
b: HLA-DRB1*0301 tag SNP.

MAF, minor allele frequency; N/A, not applicable.
doi:10.1371/journal.pgen.0030192.t001
Common family-based tests of LD, such as those used in this study (Genehunter), measure transmission distortion based on allele counts rather than genotype counts; the former has been shown to be more powerful under additive models, while the latter has greater power under recessive or dominant genetic models [27]. The genotype-pedigree disequilibrium test (geno-PDT) determines LD between a locus genotype and disease by comparing genotypes that are transmitted from parent to proband with those that are not [27]. We used the geno-PDT to assess genotypic risk for our class II and class III association signals: HLA-DRB1 and the SNP rs419788. In the case-control analysis for HLA-DRB1, ORs with 95% CI were calculated and Fisher’s exact test employed to assess statistically significant differences between HLA-DRB1 genotypes in lupus probands and healthy controls. For HLA-DRB1, the alleles were coded as follows: HLA-DRB1*0301, HLA-DRB1*1501, HLA-DRB1*X where X represents all HLA-DRB1 alleles other than HLA-DRB1*0301, and HLA-DRB1*1501. We included HLA-DRB1*1501 in the analysis, even though we find no allelic association in our cohort, because previous studies have shown a greater risk for lupus in individuals who are compound heterozygotes for HLA-DRB1*0301- and HLA-DRB1*1501-containing haplotypes [25,28]. Overall the results are consistent with a dominant effect from HLA-DRB1*0301 (Table 2) and a dose-dependent (additive) effect from rs419788-T (Table 3). Specifically, both case-control and geno-PDT demonstrate that there is no dose-dependent increase in disease risk for HLA-DRB1*0301. Rather, it appears that the presence of a single copy of HLA-DRB1*0301 alone is sufficient to increase susceptibility to disease. Moreover the 0301/X genotypes constitute the greatest risk in our cohort rather than the 0301/1501 heterozygotes. Genotypes containing HLA-DRB1*1501 in the absence of HLA-DRB1*0301 revealed no significant association in our cohort.

All three rs419788 genotypes demonstrated significant association in our lupus families (Table 3). The common CC
Characterization of HLA-DRB1*0301 Risk Haplotype

Next, we wanted to further delimit the MHC class II association signal that we have detected at HLA-DRB1. We used phased parental genotype data to compare the allelic composition of HLA-DRB1*0301-bearing haplotypes that were transmitted (T) to affected probands to those that were not transmitted (or untransmitted, UT) with the aim of identifying differences that could delineate the lupus susceptibility interval(s) arising from this haplotype (summarized in Figures 2A, 2B, and S2). We observed a striking difference between transmitted and untransmitted chromosomes within the class II region: nearly all transmitted HLA-DRB1*0301 haplotypes (99%) are identical across a 180 kb region defined by eight SNPs, whereas the corresponding region within untransmitted HLA-DRB1*0301 haplotypes exhibits significant recombination. These data strongly suggest the existence of a risk haplotype that, interestingly, contains only three expressed genes: HLA-DRB1, HLA-DQA1, and HLA-DQB1. Furthermore, we can confidently define the allelic composition of this risk haplotype, as these three genes are in strong LD and occur in one common haplotype in Caucasians: HLA-DRB1*0301-HLA-DQA1*0501-HLA-DQB1*0201. Thus, we hypothesize that the specific combination of all three alleles is required to confer disease risk in lupus or that disease susceptibility lies with either HLA-DRB1*0301 or the HLA-DQ alleles. We do not have sufficient numbers of recombinant chromosomes in this risk region to further delimit this signal: 2/176 (1.1%) transmitted HLA-DRB1*0301 haplotypes are recombinant at HLA-DQA1-HLA-DQB1; 3/178 (1.7%) transmitted haplotypes identical across HLA-DQA1-HLA-DQB1 do not possess HLA-DRB1*0301.

The composite relative extended haplotype homozygosity (REHH) versus frequency plot for UK SLE; Utah residents with ancestry from northern and western Europe (CEPH); and Yoruba in Ibadan, Nigeria (Yoruba) populations is shown in Figure 3A. We can only comment on evidence for positive selection in CEPH individuals, as we have used this population alone to assess background variation on Chromosome 6. The SLE and Yoruba cohorts are shown for comparative purposes. We find no evidence of positive selection for HLA-DRB1*0301 in the CEPH population. However, this allele is enriched in our lupus cohort (21% of parental chromosomes) and displays greater extended homozygosity when compared with HLA-DRB1*0301-bearing haplotypes in CEPH and Yoruba. Hence, the HLA-DRB1*0301 allele in lupus is observed as an outlier on the plot when compared to background variation in CEPH. These data support our previous observations (outlined above) of the highly conserved nature of HLA-DRB1*0301 haplotypes in lupus. In addition, the haplotype bifurcation plots centered on HLA-DRB1*0301 for UK SLE, CEPH, and Yoruba populations in Figure 3B illustrate preservation of the common HLA-DRB1*0301 haplotype in CEPH and UK SLE, while that seen in the Yoruba is significantly different. The class II regions of all three populations are essentially identical across our chosen SNPs; the main differences lie in class III. The difference in African populations in the class III region is one possible explanation for the lack of evidence for an association between HLA-DRB1*0301 and SLE in African or African American populations. However, HLA-DRB1*0301 has a lower frequency (~7%–10%) in African populations compared with Europeans (~13%), and the number of HLA association studies conducted in African populations is very limited.

Characterization of Class III Region Risk Haplotype

Our data reveal a second independent signal at the MHC in SLE arising from the T allele of SNP rs419788 in intron 6 of the class III gene, SKIV2L. Further evidence supporting the independence of the rs419788-T and HLA-DRB1*0301 alleles is provided by the moderate LD between these two variants (r² = 0.24) coupled with our data demonstrating that only 47% of rs419788-T allele-bearing haplotypes contain HLA-DRB1*0301.

The structure and composition of T and UT haplotypes anchored at rs419788-T were essentially identical (Figures 2C, 2D, and S2), and hence not informative in delimiting our class III signal. Therefore, we examined the LD structure around our associated class III SNP to better define our disease risk interval. In our lupus dataset the rs419788-T allele resides on three of seven haplotypes present within a large block of six SNPs exhibiting strong LD. This haplotype block encom-
Figure 2. Structure of Transmitted and Untransmitted HLA-DRB1*0301 and rs419788-T Allele Haplotypes

Haplotype bifurcation plots were constructed using 120 randomly selected parental chromosomes from each of the four datasets for comparative purposes except (B), where there were only 90 chromosomes in the entire dataset: (A) T HLA-DRB1*0301 haplotypes, (B) UT HLA-DRB1*0301 haplotypes, (C) T rs419788-T allele haplotypes, and (D) UT rs419788-T allele haplotypes. The allelic composition of the most common haplotype in each subset is shown: the core allele is represented as a dark blue double bar indicating haplotypes to the right and to the left of the core; otherwise, the common haplotype is depicted by dark grey bars. In parts (A) and (B), the rs419788-T allele in class III that shows association independent of HLA-DRB1*0301 in our cohort is indicated in green, while in parts (C) and (D), the allele HLA-DRB1*0301 is shown in green. The key difference between HLA-DRB1*0301 T and UT haplotypes lies within the class II region of the MHC. All HLA-DRB1*0301 T haplotypes are identical across a 180 kb region defined by eight SNPs (light blue), whereas the corresponding region within UT HLA-DRB1*0301 haplotypes exhibits significant recombination. This conserved class II interval encompasses only three expressed genes: HLA-DRB1, HLA-DQA1, and HLA-DQB1. Given the strong LD exhibited by HLA-DRB1*0301 haplotypes, the allelic composition of this risk region is known to be HLA-DRB1*0301-HLA-DQA1*0501-HLA-DQB1*0201. Both T and UT rs419788-T allele haplotypes show similar structure overall. The rs419788-T allele is clearly present on HLA-DRB1*0301 and non-HLA-DRB1*0301-containing haplotypes, lending credence to our observation that rs419788-T or another variant in LD with it constitutes an association signal independent of HLA-DRB1*0301 in our UK SLE cohort. (A) 120 from a total of 176 parental chromosomes, (B) 90 from a total of 90 parental chromosomes, (C) 120 from a total of 284 parental chromosomes, and (D) 120 from a total of 182 parental chromosomes.

doi:10.1371/journal.pgen.0030192.g002
(A) REHH vs. Hapmap Allele Frequency for Chromosome 6 in UK SLE, CEPH and Yoruban populations

(B) Comparison of DRB1*0301 haplotypes in (i) UK SLE, (ii) CEPH and (iii) Yoruban populations
passes roughly 270 kb containing class III genes from SLC44A4 to AGER, including the RCCX module. Next, we analyzed the haplotype block structure of this region in CEPH families using SNP data dumped from the International HapMap Project (http://www.hapmap.org/). The greater density of SNP typing available in the HapMap CEPH population compared to our current UK SLE map allowed us to potentially refine our signal by exploring correlations between our associated SNP and those surrounding it. Analysis of these data (Figure 4) suggests the presence of short-range LD around our associated variant, rs419788, in CEPH families, encompassing approximately 40 kb of the genome which includes the five genes: complement factor B (CFB), RD RNA binding protein (RDBP), SKIV2L, dom-3...
homolg Z (C. elegans) (DOM3Z), and serine/threonine kinase 19 (STK19), and does not include the complement C4 locus. Furthermore, assessment of marker association in our lupus dataset demonstrates that after conditioning for HLA-DRB1*0301, the only markers that retain association signals are telomeric of SKIV2L, suggesting that complement C4, which is centromeric to this gene, may not be responsible for our independent class III signal.

Subphenotype Analysis

In order to gain further insight into disease pathogenesis, we examined common lupus subphenotypes. Such subsets are more homogeneous than lupus per se and thus maybe enriched for specific predisposing variants. In addition, one might expect a close association between MHC class II alleles and autoantibody subsets in lupus if these are indeed causal variants, given their role in antigen presentation and subsequent humoral immunity. We therefore tested our two main MHC association signals, HLA-DRB1*0301 and rs419788, for association with renal disease and autoantibody subsets in our lupus cohort.

We found that HLA-DRB1*0301 was associated with the presence of anti-Ro and anti-La antibodies in our UK SLE cohort, with the latter showing the greatest evidence of association (anti-La nominal p < 0.001 compared with anti-Ro nominal p < 0.025). We found no association of HLA-DRB1*0301 with renal disease or any other autoantibody subsets in our dataset (see Table S4 for detailed results).

Genotypes of the SNP rs419788 were not associated with any of the tested lupus subphenotypes after controlling for the effect of HLA-DRB1*0301 (unpublished data).

Discussion

We present the first family-based SNP association study of the MHC in SLE. We have genotyped 69 markers (HLA-DRB1 and 68 SNPs) across 2.4 Mb of the MHC, encompassing class III and class II, in a cohort of 314 UK Caucasian SLE trios. Transmission disequilibrium testing of these data has shown predominant association with the alleles HLA-DRB1*0301 and rs419788-T, together with 12 other MHC SNPs. Moreover, using conditional analyses, we have shown that the two primary signals of association at the MHC are independent of each other. Specifically, one signal arises from HLA-DRB1*0301 in class II and the other from the T allele of SNP rs419788 in the class III gene SKIV2L.

Examination of bifurcation plots for T and UT HLA-DRB1*0301-containing haplotypes has enabled delineation of our class II association signal to a 180 kb region encompassing HLA-DRB1*0301-HLA-DQA1*0501-HLA-DQB1*0201. These data substantially refine that previously published by Graham et al. in 2002 [25], where the lupus susceptibility interval within HLA-DRB1*0301-containing haplotypes could only be delimited to a 1 Mb region encompassing class II and class III. The precise causal variant(s) within this region remains to be determined, as the three implicated alleles exhibit strong LD with few recombination events separating them (two out of 176 transmitted HLA-DRB1*0301 chromosomes in our dataset). However, all three allelic variants represent attractive functional candidates in lupus susceptibility for their role in antigen presentation and stimulation of the adaptive immune response.

Our association of HLA-DRB1*0301 with lupus concurs with published data in Caucasian cohorts and is well established [16]. While our lack of association with HLA-DRB1*1501 and HLA-DRB1*0801 is consistent with previous data from the UK [29], Spain [30], the Netherlands [31], Sweden [32], Mexico [33], and the US [34], it conflicts with that of other US groups [25,35]. Interestingly, we demonstrate a trend, though not statistically significant, for undertransmission of HLA-DRB1*0701—a result also observed in prior UK and Canadian lupus studies [29,36]. Moreover, a negative association of HLA-DRB1*0701 has been reported in other autoimmune diseases including Graves disease [37,38], type 1 diabetes [39], and rheumatoid arthritis [40].

It appears that the conflicting results between UK SLE and previous US (Minnesota [MN]) [25] SLE data stem from differences in HLA-DRB1 allele frequency in the probands of each cohort. The reason for this is unclear. A comparison between UK and MN SLE cohorts (Table 4) reveals that UK SLE cases are enriched for HLA-DRB1*0301 but not HLA-DRB1*0801 or HLA-DRB1*1501 when compared to a UK control population. In contrast, MN SLE cases are enriched for HLA-DRB1*0301-DQB1*0201, DRB1*0801-DQB1*0402, and DRB1*1501-DQB1*0602 inferred haplotypes when compared to MN controls [25]. There is no statistically significant difference in the aforementioned HLA class II alleles/haplotypes between UK and MN control populations that could account for the disparity seen in the respective lupus

<table>
<thead>
<tr>
<th>HLA-DRB1 Allele</th>
<th>UK SLE Allele Frequency (%)</th>
<th>UK Control Allele Frequency (%)</th>
<th>MNSLE Inferred HLA-DRB1-DQB1 Haplotype Frequency (%)</th>
<th>MNSLE Haplotype Frequency (%)</th>
<th>MN SLE Control Haplotype Frequency (%)</th>
<th>MN Control Allele Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-DRB1*0301</td>
<td>27.7</td>
<td>12.7</td>
<td>0301-0201</td>
<td>17.3</td>
<td>9.8</td>
<td>13.7</td>
</tr>
<tr>
<td>HLA-DRB1*0801</td>
<td>2.2</td>
<td>2.0</td>
<td>0801-0402</td>
<td>3.2</td>
<td>2.2</td>
<td>3.2</td>
</tr>
<tr>
<td>HLA-DRB1*1501</td>
<td>14.4</td>
<td>14.7</td>
<td>1501-0602</td>
<td>21.8</td>
<td>14.2</td>
<td>12.4</td>
</tr>
</tbody>
</table>

Table 4. Comparison of HLA-DRB1 Allele/Haplotype Frequencies in UK SLE and US (Minnesota) SLE Cohorts

*aSource: current publication.

*bSource: http://www.allelefrequencies.net.

*cSource: [25].

MN, Minnesota; n, number of chromosomes.
doi:10.1371/journal.pgen.0030192.004
cohorts. Differences in disease severity and subphenotype frequency between the two populations could account for the observed discrepancy. From the limited data available we found that the presence of renal disease appears to be similar in both cohorts (UK SLE 36% compared with MN SLE 40%), while the gender ratios are significantly different (female: male UK SLE 11:1 compared with MN SLE 57:1, Chi square p value < 0.001). We were unable to compare other lupus subphenotypes. Furthermore, closer inspection of these data reveals that microsatellite-inference of HLA-DRB1 alleles in the MN SLE dataset may underestimate the frequency of HLA-DRB1*0301 and overestimate that of HLA-DRB1*1501, thus diminishing the effect of the former and enhancing that of the latter. It is also possible that the MN SLE cohort shows greater racial heterogeneity in comparison to our UK SLE cohort, despite both being characterized as Caucasian.

Previous studies have demonstrated increased risk for lupus in individuals carrying particular combinations of microsatellite-inferred HLA-DRB1-HLA-DQB1 haplotypes [25,28]. The highest risk genotype was found to be the compound heterozygote HLA-DRB1*0301-DQB1*0201/HLA-DRB1*1501-DQB1*0602, while HLA-DRB1*0301-DQB1*0201- containing genotypes demonstrated a dose-dependent effect in increasing lupus susceptibility [25,28]. In the present study, we have examined genotypic risk at the classically typed HLA-DRB1 locus and in contrast to the aforementioned data of Graham et al. [25,28] we have shown a likely dominant effect of the associated allele, HLA-DRB1*0301. The case-control and family-based analyses for HLA-DRB1 also show the greater power of the former to detect significant association (Table 2). Specifically, all genotypes containing HLA-DRB1*0301 show increased transmission to lupus probands; however, homozygotes show no greater risk compared with heterozygotes, as would be expected under additive or multiplicative models. Thus, a dominant model of inheritance, requiring the presence of a single copy of the disease-predisposing variant alone, likely underlies the susceptibility conferred by HLA-DRB1*0301 in UK SLE. Such a model would fit an antigen presentation hypothesis where susceptible individuals carrying an HLA-DRB1*0301 allele are able to present auto-antigens to CD4+ lymphocytes, thus stimulating an autoimmune response. The differences between our UK SLE and the previously published US SLE data may reflect disease, ethnic, and haplotypic heterogeneity.

Interestingly, analysis of genotypic risk at the associated class III marker, rs419788, suggests an additive (dose-dependent) pattern of inheritance for the rare T allele, where one copy confers a low risk of disease and two copies results in greater susceptibility. The different inheritance patterns for our class II and class III association signals provide further evidence for their independence.

A variety of HLA-DR and HLA-DQ alleles have been associated with autoimmune subsets in ethnically diverse populations of lupus. The strongest associations have been demonstrated between anti-Ro and anti-La antibodies and HLA-DR3 and HLA-DQ2 (HLA-DQB1*0201), which are in strong LD [41–45] in case-control studies. Here, we confirm the association of HLA-DRB1*0301 with anti-Ro and anti-La antibody production in our family-based cohort.

Examination of LD structure around our second independent association, rs419788-T in class III, coupled with the results of our conditional analysis, suggests that this signal could also be delimited to a relatively narrow genomic interval of about 40 kb given further SNP mapping in our cohort. This region includes the genes CFB, RDBP, SKIV2L, DOM3Z, and STK19, but does not include complement C4. Thus, complement C4 null alleles, which have been implicated in lupus pathogenesis, may not be responsible for our class III signal. We conclude, therefore, that our family-based mapping study has potentially revealed a hitherto unknown lupus susceptibility interval in the class III region of the MHC. However, we cannot conclusively exclude association at complement C4RCCX without direct determination of C4 polymorphism/copy number in our cohort.

With respect to the genes implicated in our study, CFB is a vital component of the alternate complement pathway and dis-regulation may clearly affect the inflammatory response [46]. RD and Skiv2L are proteins potentially involved in RNA processing. The RD protein forms part of a negative elongation factor (NELF) complex that represses RNA polymerase II transcript elongation, while Skiv2L is a DEAD box protein with possible function as an RNA helicase. The function of Dom3z is currently unknown, although the homologous yeast protein binds nuclear exonuclease. Moreover, its ubiquitous expression suggests a housekeeping role. STK19 is a protein kinase of unknown function with primary nuclear localization [47]. Interestingly, RDBP and SKIV2L are found to be highly expressed in T lymphocytes, B lymphocytes, and dendritic cells (SymAtlas, http://symatlas.gnf.org/SymAtlas).

A number of studies have demonstrated conflicting evidence for and against association with various TNF locus polymorphisms in SLE [48]. A recent meta-analysis of the TNF-308G/A promoter polymorphism in SLE [48] revealed evidence of association for the minor allele (A) in European populations; however, this study did not account for LD with class II alleles. On conditioning our dataset for HLA-DRB1*0301, we find that the TNF promoter signal is lost, suggesting that this association is not independent and is due to LD with HLA-DRB1*0301 (or another variant in LD with HLA-DRB1*0301).

In summary, we have found association with two distinct and independent variants within the class II (HLA-DRB1*0301) and class III (SKIV2L) regions of the MHC in UK SLE trios. We can delimit our class II signal in lupus to three genetic variants (HLA-DRB1*0301-HLA-DQA1*0501-HLA-DQB1*0201) that may confer disease risk in combination or as separate signals. Our class III signal importantly excludes independent association at the TNF promoter polymorphism TNF-308G/A and potentially provides a novel locus for further study.

Materials and Methods

Study cohorts. SLE families. The cohort comprises 314 complete SLE trios (that is, mother, father, and affected lupus proband) collected as previously described [49]. All study participants are European Caucasian on the basis of grandparental origin. All 314 lupus probands (288 female, 26 male) fulfill the revised American College of Rheumatology (ACR) criteria for SLE [50], 36% of whom have a diagnosis of lupus nephritis. Written consent was obtained from all study participants and ethical approval for this study was obtained from the Multi-Centre Research Ethics Committee (MREC 2 June 1998).

Healthy controls. The control population for the HLA-DRB1 genotypic risk case-control analysis constitutes 1,667 healthy males of Northern European origin. The individuals are potential hemato-
analyses was based on permutation testing (10,000 permutations). The were constructed and permutation testing performed using Haplo- rate. The mean call rate for all markers post-quality control was 94% monomorphic in our dataset, four SNPs yielded low genotyping measures (see Table S1 for details). In summary, one SNP was to include these markers in the final analysis.

Uniform opinion in the community regarding the inclusion or Hardy–Weinberg equilibrium (HWE), which may reflect an unde- 

region between HLA-DRB9 (http://pngu.mgh.harvard.edu/purcell/plink/) [59]. Conditional regression analyses were undertaken using WHAP [60]. The geno-PDT was performed using PDT version 5.1 with default settings [27]. The HLA-DRB1 alleles were coded into three groups for the geno-PDT and the case-control analysis: HLA-DRB1*0301, HLA- DRB1*0101 and all HLA-DRB1 alleles other than HLA-DRB1*0301 or HLA-DRB1*0101. The HLA-DRB1*0101 code in the healthy controls represents the allele string HLA-DRB1*1501/1502/1504/1506, as described previously. The HLA-DRB1*0101 code in the lupus probands represents the alleles HLA- DRB1*0101 (out of 942 UK SLE trios and *1502 alleles) and HLA- DRB1*0302 (*1501 and *1502 alleles), as *1504 and *1506 were not present in this population. Fisher’s exact test was used to assess significance of association in the case-control analysis.

Subphenotype analysis. We looked for association of the HLA-DRB1*0301 allele with autoantibody subsets and renal disease in our cohort using the Chi-square test. We compared cases with and without the subphenotype of interest with DRB1*0301 homozygosity, heterozygosity, combined homozygosity and heterozygosity, and non- DRB1*0301 status. We performed the same analyses for homozygous and heterozygous genotypes of the associated SNP rs149788. The autoantibody subsets compared were anti-C1q, IgG, and IgM anti-cardiolipin antibodies (AACL and ACLM), anti-Ro, anti-La, anti-RNP, anti-Sm, and anti-dsDNA.

Delineation of associated MHC haplotypes and evidence for positive selection. We looked for positively selected alleles in our data with the long-ranged selective scan. To determine the extended haplotype homozygosity (EHH), previously described by Sabeti et al. [26]. Essentially, such an analysis allows assessment of positive selection by mining datasets for high frequency extended haplotypes in comparison to the other core haplotypes at a locus. EHH is defined as the probability that two randomly chosen chromosomes carrying the core haplotype of interest will be identical by descent (homozygosity at all SNPs) for the entire interval from the core to a distance x. The REHH is the ratio of the EHH on the tested core haplotype compared with the combined EHH of all the other core haplotypes at the region excluding the tested core; as such, REHH accounts for local variation in recombination rate while EHH does not [26].

The program emphase was employed to assign the phase of parental genotype data and reconstruct missing information. Emphase is a simple phaser similar to the phaser of Excoffier and Slatkin [61]. It is very fast, especially on large datasets, and sufficiently accurate for most genetic applications. EHH analysis was performed on the phased parental data using the software program SWEEP (http://www.broad.mit.edu/mpg/sweep/index.html).

Haplotypes and haplotype tagging SNPs from a subsequent high-resolution MHC SNP map [51] and allele frequencies, so although we have used a male control

SUPPORTING INFORMATION

Figure S1. OR Plot for Associated MHC Markers in 314 UK SLE Trios OR with 95% CI and marker name in genomic order (class I to class II, left to right) are indicated on the vertical and horizontal axes respectively. The greatest ORs are seen in the class III and class II regions. Found at doi:10.1371/journal.pgen.0030192.sg001 (42 KB PDF).

Figure S2. Structure of All Transmitted and Untransmitted HLA-DRB1*0301 and rs419788-T Allele Haplotypes Haplotype bification plots constructed for all (A) transmitted (T) HLA-DRB1*0301 haplotypes, (B) untransmitted (Ut) HLA- DRB1*0301 haplotypes, (C) transmitted.ut*0301 haplotypes, and (D) untransmitted rs419788-T allele haplotypes. The allelic composition of the most common haplotype in each subset is shown: the core allele is represented as a dark blue double bar indicating
haplotypes to the right and to the left of the core; otherwise, the common haplotype is depicted by dark grey bars. In parts (A) and (B), the rs419788-T allele in class III, which shows association independent of HLA-DRB1*0301 in our cohort, is indicated in green, while in parts (C) and (D), the allele HLA-DRB1*0301 is shown in red. The key difference between HLA-DRB1*0301 T and UT haplotypes lies within the class II region of the MHC. All HLA-DRB1*0301 T haplotypes are identical across a 180 kb region defined by eight SNPs (light blue), whereas the corresponding region within UT HLA-DRB1*0301 haplotypes exhibits significant recombination. This conserved class II interval encompasses only three expressed genes: HLA-DRB1, HLA-DQA1, and HLA-DQB1. Given the strong LD exhibited by HLA-DRB1*0301 haplotypes, the allelic composition of this risk region is known to be HLA-DRB1*0301- HLA-DQA1*0501-HLA-DQB1*0201. Both T and UT rs419788-T allele haplotypes show similar structure overall. The rs419788-T allele is clearly present on HLA-DRB1*0301 and non-HLA-DRB1*0301-containing haplotypes, lending credence to our observation that rs419788-T or another variant in LD with it constitutes an association signal independent of HLA-DRB1*0301 in our UK SLE cohort.

(A) total of 176 parental chromosomes, (B) total of 90 parental chromosomes, (C) total of 284 parental chromosomes, and (D) total of 182 parental chromosomes.

Accepted for publication 21 October 2007

References

number variation and associated polymorphisms of complement compo-
ment C4 in human systemic lupus erythematosus (SLE); low copy number is a
risk factor for and high copy number is a protective factor against SLE
Visualizing human leukocyte antigen class II risk haplotypes in human
Detecting recent positive selection in the human genome from haplotype
Genotype-based association test for general pedigrees: the genotype-PDT.
Specific combinations of HLA-DR2 and DR3 class II haplotypes contribute
graded risk for disease susceptibility and autoantibodies in human SLE. Eur
tumour necrosis factor alpha, and lymphotoxin alpha gene haplotype
associations with serological subsets of systemic lupus erythematosus. Ann
(2000) TNF-308A and HLA-DR3 alleles contribute independently to
134.
DR, HLA DQ, C4A, FcgammaRlla, FcgammaRllb, MBL, and IL-1ra allelic
variants in Caucasian systemic lupus erythematosus patients suggests an
effect of the combined FcgammaRlla R/R and IL-1ra 2/2 genotypes on
D, Alcocer-Varela J, et al. (1998) Haplotype distribution of class II MHC
genes in Mexican patients with systemic lupus erythematosus. Scand J
Rheumatol 27: 373–376.
34. Uribe AC, McGwin G Jr, Revellie JD, Alarcon GS (2004) What have we
learned from a 10-year experience with the LUMINA (Lupus in Minorities:
324.
Analysis of the association of HLA-DRB1, TNFalpha promoter and TNFFR2
(TNFRSF1B) polymorphisms with SLE using transmission disequilibrium
class II HLA antigens in systemic lupus erythematosus. Lupus 8: 466–470.
37. Chen QY, Huang W, She JX, Baxter F, Volpe R, et al. (1999) HLA-DRB1*08,
DRB1*03/DRB3*0101, and DRB3*0202 are susceptibility genes for Graves’
disease in North American Caucasians, whereas DRB1*07 is protective. J
Clin Endocrinol Metab 84: 5182–5186.
Regression mapping of association between the human leukocyte antigen
Both DQA1 and DQB1 genes are implicated in HLA-associated protection from
Graves’ disease in Malaysian Chinese patients with systemic lupus erythema-
Polymorphisms of HLA class II genes and autoimmune responses to Ro/SS-
HLA class II DNA typing in a large series of European patients with
systemic lupus erythematosus: correlations with clinical and autoantibody
targeted disruption of the murine complement factor B gene resulting in
loss of expression of three genes in close proximity, factor B, C2, and
Analysis of a high-throughput yeast two-hybrid system and its use to
predict the function of intracellular proteins encoded within the human
371.
45. Russell AI, Cunningham-Graham DS, Shepherd C, Robertson CA,
influences gene expression and predisposes to systemic lupus erythema-
46. Tan EM, Cohen A.S., Fries J.F., Masi A.T., McShane D.J., Rothfield N.F.,
the classification of systemic lupus erythematosus. Arthritis Rheum 25:
1271–1277.
47. Walsh EC, Mather KA, Schaffner SF, Farwell L, Daly MJ, et al. (2003)
An integrated haplotype map of the human major histocompatibility
high-resolution HLA and SNP haplotype map for disease association
studies in the extended human MHC. Nat Genet 38: 1166–1172.
MassARRAY technology for high throughput genotyping. Adv Biochem
Eng Biotechnol 77: 57–74.
database—a sequence database for the human major histocompatibility
genotype incompatibilities in linkage analysis. Am J Hum Genet 65: 259–
266.
52. Spielman RS, Ewens WJ (1996) The TDT and other family-based tests for
54. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haplview: analysis and
55. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, et al. (2007)
PLINK: a toolset for whole-genome association and population-based
56. Purcell S, Daly MJ, Sham PC (2007) WHAP: haplotype-based association
58. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haplview: analysis and
59. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, et al. (2007)
PLINK: a toolset for whole-genome association and population-based
60. Spielman RS, Ewens WJ (1996) The TDT and other family-based tests for
62. Barrett JC, Fry B, Maller J, Daly MJ (2005) Haplview: analysis and
63. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, et al. (2007)
PLINK: a toolset for whole-genome association and population-based
64. Purcell S, Daly MJ, Sham PC (2007) WHAP: haplotype-based association