European Journal of Human Genetics (2007) 15, 823–830
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ARTICLE

Specific combinations of HLA-DR2 and DR3 class II haplotypes contribute graded risk for disease susceptibility and autoantibodies in human SLE

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The human leukocyte antigen (HLA) Class II antigen presentation alleles DR and DQ are associated with susceptibility to systemic lupus erythematosus (SLE) and the production of lupus-related autoantibodies. Here, we explore the effect of different combinations of Class II risk haplotypes in a large, multi-center collection of 780 SLE families. Haplotypes bearing the DRB1*1501/DQB1*0602 (DR2) and DRB1*0301/DQB1*0201 (DR3) alleles were present in nearly two-thirds of SLE cases and were significantly associated with disease susceptibility in both family-based and case-control study designs. DR3-containing haplotypes conferred higher risk for disease than DR2, and individual homozygous for DR3 or compound heterozygous for DR3 and DR2 showed the highest risk profile. DR2 haplotypes were also found to be associated with antibodies to the nuclear antigen Sm, and, as previously observed, DR3 genotypes were associated with Ro and La autoantibodies. Interestingly, SLE cases and unaffected family members who were DR2/DR3 compound heterozygotes showed particularly strong risk of developing antibodies to Ro, and were enriched for La and Sm. These data provide convincing evidence that particular combinations of HLA Class II DR2 and DR3 haplotypes are key determinants of autoantibody production and disease susceptibility in human SLE.
European Journal of Human Genetics (2007) **15,** 823–830; doi:10.1038/sj.ejhg.5201827; published online 4 April 2007

Keywords: systemic lupus erythematosus; human leukocyte antigen; association study

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Received 4 April 2006; revised 12 January 2007; accepted 27 February 2007; published online 4 April 2007

Introduction

Systemic lupus erythematosus (SLE, MIM :152700) is a systemic, inflammatory autoimmune disease that affects ~275 000 people in the US (0.1% population prevalence),[1] and exhibits a strong gender bias (9:1, female/male ratio). Lupus is clinically heterogeneous, and patients often

experience a progressing cycle of disease flares and remissions. Nearly half of cases will manifest severe complications of the disease, which may include glomerulonephritis and compromised renal function, and/or neurologic symptoms such as seizures, psychiatric symptoms, peripheral neuropathies or stroke. Despite advances in treatment, the mortality rate of SLE patients remains three times that of matched controls.[2]

SLE is thought to result from complex interactions among environmental, hormonal, and genetic factors.[3–6] A genetic component for SLE is well-established and is supported by twin studies, the familial aggregation of disease, and the identification of several confirmed SLE risk genes.[7–11] The first genetic association described for SLE was with the human leukocyte antigen (HLA) region.[9] The HLA region is contained within a 3.6 Mb interval located on human chromosome 6p21.3 that encodes over 200 genes, many with known roles in immunity.[12,13] The telomeric Class I region contains the ubiquitously expressed HLA-A, -B, and -C genes, which present antigenic peptides to CD8+ T cells. The centromeric Class II region contains the highly polymorphic HLA-DR, -DQ and -DP genes. These molecules are expressed at high levels on antigen-presenting cells, such as dendritic cells and B cells and display peptides for CD4+ helper T cells. The Class III region lies between the Class I and II regions and contains many immune-related genes, including the cytokines tumor necrosis factor-α and lymphotoxin-α and the complement components C2, C4 and Factor B.

In addition to the importance of the HLA for transplantation tolerance,[14] genes within the region are also major contributors to the risk for autoimmunity.[15] For instance, Class I alleles are associated with psoriasis (Cw6) and ankylosing spondylitis (B27), whereas HLA Class II alleles are associated with rheumatoid arthritis (DRB1*0401 – DR4), celiac disease (DQA1*0501, DQB1*0201), multiple sclerosis (DRB1*1501 – DR2), IgA deficiency (DRB1*0301 – DR3), autoimmune thyroid disease (DR3), type 1 diabetes mellitus (DR3, DR4) and SLE (DR2, DR3).

Antibodies specific for nucleic acids and nuclear autoantigens are a hallmark of SLE, and are involved at many levels in disease pathogenesis.[16,17] At least 4% of individuals in the general population show elevated serum titers of anti-nuclear antibodies (ANAs) in the absence of rheumatic disease, and the presence of ANAs may increase the risk for SLE as much as 40-fold.[18] Of interest, nuclear autoantibodies such as the Ro and La RNA-binding proteins can be detected in the serum of many SLE cases well before the onset of clinical symptoms.[18]

Association studies in SLE populations of European descent have generally concluded that haplotypes bearing DR3 are associated with SLE susceptibility, whereas haplotypes bearing DR2 appear to be more weakly associated (reviewed in references[19,20]). In a recent study, we demonstrated that the disease-associated HLA-DR2 containing haplotype is localized to a 500 kb interval that includes the DR and DQ genes and two genes of unknown function.[21] HLA-DR3 haplotypes showed strong linkage disequilibrium, often extending >1 Mb into the Class III and I regions, such that localization of the primary genetic effect(s) on this haplotype was difficult. Anti-La and anti-Ro antibodies have consistently demonstrated association with the DR3 Class II haplotypes,[22,23] whereas the association of antibodies to other HLA alleles remains unclear.

In the present study, we genotyped HLA Class II alleles in two large, independent replication cohorts, and examined the data together with previous typing performed in 283 MN SLE families (total of 780 pedigrees). We also examined serum autoantibody profiles in 584 SLE cases for association to HLA genotypes. These data provide support for the hypothesis that DR2- and DR3-containing haplotypes, alone and in combinations, are potent contributors to genetic risk for autoantibodies and disease-susceptibility in SLE.

Materials and methods

Family collections

We studied a multi-center US Caucasian SLE family collection of 780 pedigrees, composed of 1158 SLE cases and 1545 other family members (Table 1). The clinical features of disease and demographics of these families are described elsewhere.[24–30] The combined collection consists of 283 sib-pair and trio pedigrees collected at the University of Minnesota (MN), 146 multiplex families from the Oklahoma Medical Research Foundation (OMRF) provided by the Lupus Multiplex Registry and Repository (AR12253), and 351 trio pedigrees collected at the University of California-San Francisco (UCSF). These studies were approved by the Human Subject Institutional Review Boards at each institution, and informed consent was obtained from all subjects.

Genotyping

Class II haplotypes were determined by typing three HLA region microsatellite markers (D6S2666, D6S2665,

Table 1 Composition of three independent caucasian SLE family collections

	SLE cohort			
	MN[a]	*OMRF*[b]	*UCSF*[c]	*All*
Multiplex/sib-pair pedigrees	164	146	—	310
Trio pedigrees	119	—	351	470
Total pedigrees	283	146	351	780
Affected individuals	471	325	362	1158
Unaffected individuals	466	427	652	1545
Total individuals	937	752	1014	2703

[a]University of Minnesota.
[b]Oklahoma Medical Research Foundation.
[c]University of California - San Francisco.

D6S2446) that flank the DR locus and are in tight linkage disequilibrium.[21] Our previous studies demonstrated that these markers tag Class II haplotypes with high specificity and fidelity.[21,31] The markers were genotyped in all 2703 individuals at the University of Minnesota, as described.[21] Detailed information regarding primer sequences and locations are presented in Supplementary Table S1, and allele frequency distributions are shown in Supplementary Table S2. Microsatellite marker haplotypes for the pedigrees and controls were constructed using standard parameters of PHASE v2.1.[32] Samples ($N=44$) with $>50\%$ missing data or where phase could not be confidently assigned with a probability $<50\%$ ($N=260$) were excluded from the analysis.

To verify further the ability of haplotypes constructed from the D6S2666/2665/2446 microsatellites to faithfully represent underlying HLA Class II haplotypes, we genotyped the D6S2666/2665/2446 markers in 902 Caucasian control individuals from the New York Cancer Project (NYCP) collection,[33] for whom DRB1 allele status was available as determined by oligotyping.[34] The specificity of the microsatellite haplotypes as surrogates for all of the common DRB1 alleles averaged 99.8%. The ability of the microsatellite haplotypes to predict correctly the DRB1 allele (as determined by oligotyping) averaged 99.0% and for the microsatellite haplotypes bearing DRB1*1501/DQB1*0602 (DR2), DRB1*0301/DQB1*0201 (DR3) and DRB1*0801/DQB1*0402 (DR8) the positive predictive value was 96.7, 100 and 100% respectively.

In the NYCP controls, the microsatellite analysis identified a few rare marker combinations for HLA- DRB1*02 and DRB1*03 (as defined by low-resolution oligotyping) (Supplementary Table S3). The rare HLA-DRB1*02 haplotypes and two of the HLA-DRB1*03 haplotypes showed no evidence or trend of being enriched in SLE cases, and were excluded from the current analysis. One of the rare HLA-DRB1*03 associated marker haplotypes was found at an allele frequency of 1.72% of cases and 0.89% of controls ($P=0.05$). This variant haplotype shares the identical alleles at D6S2666 and D6S2665 with the common HLA-DRB1*0302 haplotype, but differs by three repeat units at D6S2446 and likely represents the DRB1*0302 allele. Although showing borderline evidence for association, this uncommon DR3 microsatellite variant was excluded from the current analysis. The conclusions of this present study were not different when this haplotype was included in the analysis (data not shown).

Autoantibody measurements

Autoantibody data from 584 SLE patients (from 274 independent pedigrees of the OMRF and MN collections) and 533 unaffected relatives were used in the analyses. Precipitating (high-titer) levels of Abs reactive with ENAs included anti-Ro/SSA, anti-La/SSB, anti-Sm, anti-nRNP and anti-ribosomal P protein and were detected by Ouchterlony assays.[35,36] These autoantibodies were dichotomously classified as being 'present' or 'absent'. Abs reactive with anti-dsDNA were measured by *Crithidiae lucilliae* assays (Protrac Industries, Kerrville, TX, USA). All individuals with detectable anti-dsDNA Abs at a titer of 1:120 were considered positive.

Analysis

Family-based association analysis To avoid the potential bias of selecting a single case from multiplex pedigrees, a single trio or, if only one parent was available, a single dyad from each pedigree was randomly selected. The random trio/dyad selection was repeated 10 000 times. The TDT/S-TDT 1.1 program[37] (a valid test of association in trio and dyad pedigrees) was then applied to each of the pedigree files and an average of the transmissions/non-transmissions for each run was calculated. The nominal P value was calculated for the χ^2 generated from 2×2 contingency tables.

To assess further the statistical significance of the results, permutation testing was employed. Using Haploview v3.2 (http://www.broad.mit.edu/mpg/haploview/), the transmitted/untransmitted status of each haplotype was randomly permutated for 100 000 iterations and the best χ^2 value generated for each permutated dataset was recorded. The number of times the permutated χ^2 value exceeded the nominal χ^2 value was divided by the number of iterations (100 000) to generate the permutated P value.

The pedigree disequilibrium test (PDT) and genotype PDT (genoPDT) were performed using PDT v5.1 with default settings.[38,39] The PDT and TDT were performed using single microsatellite markers and microsatellite haplotypes. For several of the analyses, the microsatellite haplotypes were coded such that the 'non-risk' HLA haplotypes were grouped into a single allele (DRX) and tested for association relative to the HLA 'risk' haplotypes.

Autoantibody association analysis SLE cases or unaffected family members were stratified by HLA genotype, and the number and frequency of autoantibody positive samples was determined for each HLA genotype. The frequency of autoantibodies (Ro, La, Sm, P, RNP, the presence of >1 ENA and dsDNA) in individuals carrying an HLA 'risk' haplotype was compared with the frequency of antibodies in individuals not carrying an HLA 'risk' haplotype (DRX/DRX). Only one of 461 unaffected family members tested positive for dsDNA antibodies, therefore dsDNA antibodies were not analyzed in these individuals. The significance of the differences in autoantibody frequencies between individuals carrying the risk haplotypes and individuals not carrying a risk haplotype (DRX/DRX) was assessed by calculating the P value after correcting for familial correlation by using the empirical estimate of the variance.[40]

Results

Family-based association testing of HLA class II risk haplotypes in SLE

We examined the association of HLA Class II haplotypes in a replication cohort, comprised of two independent SLE family collections from OMRF and UCSF (total $N=497$) (Table 2). Application of the Transmission Disequilbrium Test (TDT) to the dataset confirmed that DR2- ($P=0.02$) and DR3-containing ($P=2.1\times10^{-4}$) haplotypes were associated with SLE in the replication cohort, and in the combined collection (Table 2). The DR8 haplotype was also over-transmitted, but was relatively rare in this collection and the association failed to reach statistical significance. Similar significance levels were obtained by permutation of the transmission and non-transmission status of parental chromosomes (Table 2). No other HLA class II haplotypes demonstrated a positive association with SLE (data not shown). The PDT, a global test of association appropriate for complex pedigrees, also showed highly significant transmission distortion at the Class II region; global $P=3\times10^{-5}$ for the 497 replication pedigrees, and global $P=1\times10^{-12}$ in the combined dataset of 780 pedigrees. In addition, the allele and genotype frequencies of the HLA Class II haplotypes in 780 unrelated SLE cases selected the pedigrees and 902 Caucasian controls from the NYCP[33] database were examined to estimate the effect of these alleles relative to the general population (Supplementary Table S4). From these analyses using both family-based and case-control designs, we conclude that haplotypes containing DR2, DR3 and possibly DR8 are associated with susceptibility to SLE.

Genotypic risk of HLA class II haplotypes in SLE

To address the combinatorial effects of HLA risk haplotypes and model of inheritance, we analyzed the transmission distortion of genotypes in these families using the genotype-PDT (genoPDT).[39] In the genoPDT, instead of measuring the transmission patterns of single alleles within a trio as in the TDT or PDT, the transmission of the genotype is measured. Of the four parental alleles in each trio, the two transmitted alleles comprise the transmitted genotype, whereas the two non-transmitted parental alleles are considered the non-transmitted genotype. While the allele-based PDT is a more powerful test under simple additive models, the genoPDT has more power under dominant and recessive models.[39]

Applying the genoPDT to this dataset revealed a transmitted/non-transmitted (T/NT) ratio of 1.3 for DR2/DRX (where X is any non-DR2 or non-DR3 haplotype) ($P=0.04$) and 1.4 for DR3/DRX genotypes ($P=0.03$) (Table 3). A significantly higher T/NT ratio was observed for DR3/DR3 (T/NT = 2.1, $P=0.03$) and for DR2/DR3 (T/NT = 2.3, $P=0.0001$) genotypes, consistent with a dose effect of the DR3 haplotype and an increased risk in DR2/DR3 compound heterozygotes.

Autoantibody associations with class II genotypes

We did not observe significant associations between HLA class II status and ACR criteria for SLE in the 780 SLE pedigrees (data not shown), consistent with previous studies (Harley *et al.,* 1998a). We next examined whether the identified HLA haplotypes were associated with autoantibodies in this family collection. Sera of 584 SLE cases

Table 2 Transmission disequilibrium test for HLA Class II haplotypes in 780 SLE families

	Haplotype	*T*	*NT*	*T/NT*	χ^2	*Nominal P*	*Permuted P*[a]
Original cohort[b] (283 pedigrees)	DR2	69	37	1.86	9.7	1.9×10^{-3}	0.0022
	DR3	59	30	1.97	5.4	2.1×10^{-3}	0.001
	DR8	14	7	2.00	2.3	NS	NS
	non-risk	53	121	0.44	26.6	2.5×10^{-7}	<0.00001
Replication cohort[c] (497 pedigrees)	DR2	87	59	1.47	5.4	0.02	0.058
	DR3	89	46	1.93	13.7	2.1×10^{-4}	0.0003
	DR8	13	10	1.30	0.4	NS	NS
	non-risk	80	155	0.52	23.9	1.0×10^{-6}	0.00002
Combined cohort[d] (780 pedigrees)	DR2	157	96	1.64	14.7	1.3×10^{-4}	0.0003
	DR3	149	76	1.96	23.7	1.1×10^{-6}	<0.00001
	DR8	27	17	1.59	2.3	NS	NS
	non-risk	135	279	0.48	50.1	1.5×10^{-12}	<0.00001

DR2, DRB1*1501/DQB1*0602; DR3, DRB1*0301/DQB1*0201; DR8, DRB1*0801/DQB1*0402. Haplotypes containing the indicated DRB1/DQB1 alleles were determined by microsatellite genotypes for the markers D6S2666/2665/2446 as described in the text.
T, transmitted haplotype; NS, not significant, $P>0.05$; NT, non-transmitted haplotype.
[a]Permuted P, 100 000 random permutations of transmitted and non-transmitted chromosome status, as described in Materials and methods.
[b]MN families.
[c]OMRF and UCSF families.
[d]MN, OMRF and UCSF families.

obtained from 273 independent OMRF and MN pedigrees were assayed for the presence of autoantibodies to the following extractable nuclear antigens (ENAs): Ro/SSA, La/SSB, RNP, P, and Sm. The frequency of affected individuals positive for ENAs carrying a given HLA risk genotype was compared with the frequency of affected individuals positive for ENAs who did not carry an HLA risk haplotype (Table 4).

In general, individuals carrying the DR2 and DR3 haplotypes were more likely to be positive for antibodies to several of these nuclear antigens (Supplementary Table S6). Compared with DRX/DRX cases, DR2/DRX individuals were enriched for Sm antibodies ($P=0.0022$), whereas DR3/DRX individuals had higher frequencies of antibodies to La ($P=0.0005$) and Ro ($P=0.0003$). DR2/DR3 compound heterozygotes were enriched for antibodies to La and Sm, and were three times more likely than DRX/DRX cases to exhibit antibodies to Ro ($P=2\times10^{-6}$). Nearly 60% of DR2/DR3 individuals were positive for at least one antibody to an extractable nuclear antigen, compared to 28.7% of SLE cases not carrying a risk haplotype ($P=0.0047$).

Data for anti-dsDNA antibodies, available for 449 cases from 208 pedigrees, were similarly analyzed. In DR2/DR3 compound heterozygotes, 22 of 35 individuals (62.9%) were positive for anti-dsDNA antibodies, compared with only 28.4% of DRX/DRX individuals (50 of 176, $P=8.3\times10^{-5}$). No other HLA genotype showed significant association with dsDNA antibodies.

The relatively low numbers of DR2 ($N=13$) and DR3 ($N=19$) homozygote individuals in this dataset reduced the power to assess significance of the results. However, the DR2/DR2 group was consistent with the trends observed in DR2/DRX cases (significant association of Ro and Sm), whereas the DR3/DR3 genotype showed strong association with Sm, but not with Ro or La, and a trend towards significance for association with anti-dsDNA antibodies.

Discussion

We have studied the contribution of HLA Class II haplotypes to disease susceptibility and specific autoantibody production using a large multi-center collection of SLE families (780 pedigrees) and a large control cohort

Table 3 Genotype-pedigree disequilibrium test in 517 SLE trios[a]

Genotype[b]	*T*	*NT*	*T/NT*	*Z score*	*P*
DR2/DRX	135	104	1.3	2.0	0.04
DR3/DRX	100	71	1.4	2.2	0.03
DR2/DR2	12	13	0.9	−0.2	NS
DR3/DR3	21	10	2.1	2.2	0.03
DR2/DR3	51	22	2.3	3.5	0.0001
DRX/DRX	198	297	0.7	−5.6	2.0×10^{-8}

[a]517 unrelated complete trios (genotype data available for both parents and affected offspring), corresponding to 1034 total transmitted and untransmitted genotypes.
[b]DR2, DRB1*1501/DQB1*0602; DR3, DRB1*0301/DQB1*0201; DRX, haplotype other than DR2 or DR3. Haplotypes containing the indicated DRB1/DQB1 alleles were determined by microsatellite genotypes for the markers D6S2666/2665/2446 as described in the text; T, transmitted genotype; NT, non-transmitted genotype; NS, not significant.

Table 4 Autoantibody associations with HLA Class II genotypes

	DRX/DRX *N = 230*[a] *% (N)*	*DR2/DRX* *N = 159*		*DR3/DRX* *N = 121*		*DR2/DR3* *N = 42*		*DR2/DR2* *N = 13*		*DR3/DR3* *N = 19*	
		% (N)	P-*value*[b]	*% (N)*	P-*value*	*% (N)*	P-*value*	*% (N)*	*P value*	*% (N)*	*P value*
La	5.7 (13)	6.9 (11)	NS[d]	18.2 (22)	0.0005	14.3 (6)	NS	7.7 (1)	NS	0 (0)	—
Ro	17 (39)	25.2 (40)	NS	33.9 (41)	0.0003	52.4 (22)	0.000002	38.5 (5)	0.0306	0 (0)	—
Sm	1.7 (4)	9.4 (15)	0.0022	4.1 (5)	NS	7.1 (3)	0.0731	15.4 (2)	0.0066	15.8 (3)	0.0028
P	0.9 (2)	0.6 (1)	NS	0 (0)	—[f]	0 (0)	—	7.7 (1)	0.0784	0 (0)	—
RNP	15.2 (35)	15.7 (25)	NS	9.9 (12)	NS	9.5 (4)	NS	23.1 (3)	NS	15.8 (3)	NS
ANY ENA[e]	28.7 (66)	37.1 (59)	NS	43 (52)	0.0035	59.5 (25)	0.0047	53.9 (7)	NS	15.8 (3)	NS
	N = 176	N = 119		N = 90		N = 35		N = 12		N = 17	
dsDNA[c]	28.4 (50)	31.9 (38)	NS	26.7 (24)	NS	62.9 (22)	0.000083	25 (3)	NS	47.1 (8)	NS

DRX, haplotypes other than DR2 or DR3; DR2, DRB1*1501/DQB1*0602; DR3, DRB1*0301/DQB1*0201. Haplotypes containing the indicated DRB1/DQB1 alleles were determined by microsatellite genotypes for the markers D6S2666/2665/2446 as described in text.
[a]*N,* Number of SLE individuals with indicated genotypes;
[b]*P* value, familial correlation was accounted for by using a sandwich estimator of the variance and exchangeable correlation.
[c]Individuals positive for dsDNA antibodies at serum dilutions of 1:120.
[d]Not significant ($P>0.1$).
[e]Individuals positive for 1 or more extractable nuclear antigens (ENA), defined as La, Ro, Sm, P, or RNP.
[f]Could not be calculated.

($N = 902$). Using a variety of different analytical approaches, a remarkably clear and consistent picture emerges, with the key findings as follows: (1) using family-based association testing, we have replicated earlier work suggesting that DR2- and DR3-containing Class II haplotypes confer significant risk for the development of SLE in Caucasians; (2) we show that DR3/DR3 and DR2/DR3 are particularly strong risk genotypes for SLE susceptibility; (3) we find that a single dose of DR2 elevates the probability of autoantibodies to Sm, whereas a single dose of DR3 is associated with Ro and La autoantibodies; and (4) we identify compound heterozygosity for DR2/DR3 as a common (7.5% of SLE cases) and particularly potent risk genotype for antinuclear antibodies, and show that this genotype also predisposes to autoantibodies in unaffected family members of SLE cases. Together, these data provide important new insights into the role of specific combinations of Class II risk haplotypes in SLE disease susceptibility and autoantibody targeting.

We have used an approach to genotype Class II alleles that is based on the typing of three highly polymorphic microsatellites that flank the DR and DQ loci, rather than genotyping DR by oligotyping or other sequence-based methods. We verified that this method is a highly reliable alternative to direct oligotyping by analyzing these microsatellites in a large control cohort where DR oligotyping was performed in parallel (Rodine *et al*., in preparation).

Class II genotyping by the microsatellite haplotype method is highly sensitive and specific, indicating that the lengths of the measured dinucleotide-repeat markers has remained very stable over population history. An important advantage of microsatellite-based assignment of DR is the cost savings. These three microsatellite markers can be multiplexed into a single PCR, and performed for less than $ 1.00/genotype, which is ~100-fold savings over oligotyping. Since our data suggests that the microsatellite genotyping method can be applied to all of the common Caucasian DR alleles (Rodine *et al*., in preparation), we believe that this method can be applied widely and in a cost-effective fashion to a broad range of diseases. It is important to note that the current studies have focused on the application of this typing method in Caucasians, and further work will be required to validate this method for other ethnic groups.

Compound heterozygosity and homozygosity for HLA risk alleles have been shown to confer an increased risk for several autoimmune diseases. Homozygosity for DRB1*0401 and heterozygosity for DRB1*0401/DRB1*0404 is associated with an increased risk for rheumatoid arthritis.[41] Heterozygosity for DR3/DR4 is strongly enriched in type 1 diabetes, whereas DR2 homozygosity shows strong association in multiple sclerosis.[42,43] Of interest, early studies suggested the possibility of genetic complementation between DR2/DQ6 and DR3/DQ2 haplotypes in susceptibility to lupus, as well as to Sjogren's syndrome, a disease related to SLE characterized by a high incidence of antinuclear antibodies.[44–47]

How can compound heterozygosity for DR2/DR3 or homozygosity for DR3 contribute to the lupus phenotype? Heterozygosity of HLA antigen presentation molecules confers improved resistance to infection, presumably because of the larger and more diverse antigen repertoire that can be presented by heterozygous individuals.[48,49] Similarly, individual heterozygous for the HLA Class II alleles associated with SLE may have an increased probability of displaying self-reactive antigens. Recent data suggest that breaks in tolerance to viral proteins (eg, EBNA1 protein of EBV) may be associated with subsequent cross-reactive autoimmunity to RNA-binding proteins such as Ro and Sm.[50,51] One hypothesis that warrants consideration is that the relevant viral- or self-antigens in SLE may be restricted by the disease-associated DR2 and DR3 Class II alleles (and/or their respective DQ alleles) for presentation to CD4+ T cells, thereby increasing the probability that autoimmune responses might be initiated and propagated. An alternate hypothesis is that CD4+ and CD25+ T regulatory cells, which are important for suppressing autoimmune diseases,[52] may undergo inefficient positive selection on the SLE risk Class II alleles. The low background population prevalence of antibodies to ENAs (~1%) and the progressive appearance of these antibodies before the onset of clinical symptoms suggests that loss of immunologic tolerance to these antigens marks an important transition in the road to overt disease.[18] The possibility that DR2 or DR3 Class II molecules might be able to present peptides from the relevant EBV gene products and the Ro, La and Sm autoantigens should be explored.

Our previous studies visualizing recombinations on the ancestral haplotypes carrying DR2 allowed us to localize the major genetic-risk region on this haplotype to an ~500 kb region containing both DRB1 and DQB1.[21] The only other genes in this interval are two genes of unknown function – chromosome 6 open reading frame 10 (C6orf10 - formerly known as testis specific basic protein), and butyrophilin-like family member II (BTNL2) – together with DRA (invariant), DRB3 and DRB5 (additional β chain genes present on the DRB1*0301 and DRB1*1501 haplotypes, respectively), and DQA (a different allele for each of the risk haplotypes). Further studies are required to determine whether the genetic effects on this haplotype are limited to the Class II DRB1 and/or DQB1 genes, or include these other tightly linked genes.

The extensive linkage disequilibrium on the DRB1*0301 haplotype severely limits the ability to localize the genetic effect on this haplotype. The increased risk of the DR3 haplotype relative to the DR2 haplotype makes it tempting to hypothesize that multiple genetic effects reside on this haplotype. For example, a high-expressing TNF-α allele and C4 'null' alleles, which have been suggested previously as

risk factors for SLE,[53,54] are both carried on the extended A1/B8/DR3 haplotype. At this point, caution is warranted before assigning risk to any individual gene on the DRB1*0301 haplotype, given the extensive disequilibrium observed. Typing of a dense SNP map across the entire HLA[55] in DR3+ SLE cases and controls, may allow us to tease out additional genetic contributions to SLE present on this haplotype.

Acknowledgements

We gratefully acknowledge the many patients, families and physicians who have cooperated for the purposes of genetic studies in SLE. This work was supported by grants, contracts and fellowships from the National Institute of Arthritis, Musculoskeletal and Skin Diseases, the National Institute of Allergy and Infectious Disease, the Minnesota Lupus Foundation, the Mary Kirkland Center for Lupus Research, and the Alliance for Lupus Research. These studies were performed in part at the General Clinical Research Center, Moffitt Hospital, University of California, San Francisco, with funds provided by the National Center for Research Resources (5 M01 RR00079, US Public Health Service) and at the Oklahoma Medical Research Foundation with grants awarded to JBH (NIH grants: AR12253, AI24717, AR42460, AR048094, RR024013, AI32584, and AR048940).

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