Data S3

Systemic lupus erythematosus

Systemic lupus erythematosus (SLE, MIM 152700) is the prototypic, multi-system autoimmune disease primarily affecting women of child bearing age. The aetiology of the disease is not yet understood but genetic, hormonal and environmental influences contribute to disease susceptibility. The worldwide prevalence of SLE is estimated at between 12 and 64 cases per 100,000 individuals, with a 10:1 female gender bias [1]. There is a 4-5 fold higher prevalence in non-European (Afro-Caribbean, Indo-Asian) compared with European populations [1-5]). Lupus is characterized by the production of pathogenic IgG autoantibodies to a wide spectrum of nuclear (dsDNA, histones) and cell surface antigens (antiphospholipid antibodies). The clinical manifestations of the disease are diverse and result from inflammation and damage in a variety of organs. Lupus commonly presents with arthralgia, fatigue and rash but individuals may manifest serious and life-threatening complications due to renal, cerebral and cardiopulmonary involvement.

The human MHC was first shown to be associated with SLE in 1971 when lupus probands were found to be enriched for the class I alleles, *HL-A8* (now known as *HLA-B8*) and *HLA-W15* (now known as *HLA-B15*) when compared with healthy controls [6,7]. Subsequent case-control studies focused on the classical class I and class II genes as well as the complement *C4* locus within the class III region given the association of lupus with C4 deficiency. Interestingly, the MHC has only been significantly linked to SLE in one of the twelve genome-wide linkage scans [8,9] with supporting evidence from three further studies [10-12]. However, a recent meta-analysis of linkage studies in

lupus has demonstrated evidence of significant linkage at *6p21* [13]. There is also clear evidence from approximately 100 case-control association studies that the MHC plays an important role in SLE. However, the majority of these studies have failed to encompass the entire locus and have been undertaken in small ethnically diverse cohorts utilizing a limited number of genetic markers.

The most consistent HLA associations with SLE reside with the class II genes, *HLA-DR3* (*DRB1*0301*) and *-DR2* (*DRB1*1501*) and their respective haplotypes in predominantly white populations [14]. Studies in non-white populations are inconsistent. For instance, investigation of the *HLA-DR* locus in the LUMINA study [15] revealed increased frequency of *DRB1*0301* in Caucasians and Hispanics from Texas. *DRB1*0801* was increased in Hispanics from Texas but not Puerto Rico. African Americans in the same study were found to have higher frequencies of *DRB1*1503* (*DR2*) compared to matched controls. These populations demonstrated no association with *DRB1*1501*. Other studies in African Americans have revealed no association of *DRB1* alleles with SLE [16,17]. An association with *HLA-DR4* was shown in the only study in North Indians [18]. A number of other studies in lupus have demonstrated associations among Mexican [19], Tunisian [20], Korean [21] and Thai [22] populations with *DRB1*15*; Mexican and Tunisian patients with *DRB1*0301* and *DRB3*01/03* in Jamaicans [22].

One might expect a close association between class II alleles and autoantibody subsets in lupus if these are indeed the causal variants. A variety of *HLA-DR* and *-DQ* alleles, have been associated with autoantibody subsets in ethnically diverse lupus populations. The strongest associations

have been demonstrated between anti-Ro/La antibodies and *DR3* and *DQ2* (*DQB1*0201*) which are in strong linkage disequilibrium [23-27]. Studies of individuals with the antiphospholipid syndrome and antiphospholipid antibodies in lupus show predominant association with the *DR4/DQ8* (*DQB1*0302*) haplotype as well as other class II alleles [28-30].

The MHC class I specificities, *A1* and *B8*, have been linked with SLE. However these alleles reside on the disease-associated *DR3* haplotype, AH8.1, and this effect most likely results from linkage disequilibrium.

Despite the fact that the class III region is the most gene-dense in the genome, only complement *C4* and *TNF* polymorphisms have been studied in any detail in lupus. Inherited (and acquired) deficiencies of the early classical complement components, C2, C4A, C4B, encoded within this region are associated with the development of lupus although penetrance is not complete [31]. The presence of *C4A* and *C4B* null alleles (*C4A*Q0* and *C4B*Q0*) which result in partial C4 deficiency have been associated with lupus [32,33]. However, these alleles are in strong linkage disequilibrium with specific ancestral haplotypes: *A1-B8-TNF-308A-C4A*Q0-DRB1*0301* (AH 8.1) and *A30-B18-C4B*Q0-DRB1*0301* (the “Basque” haplotype), so to date it has not been possible to establish the identity of the causal allele(s) located within these extended haplotypes in lupus. The *C4* null allele associations seem to reflect the prevalent *DRB1*0301* haplotype in the population under study. In southern European populations the *C4B* null allele in LD with the *A30-B18-DRB1*0301* haplotype shows association with SLE [33-35] while the *C4A* null allele in LD with the *A1-B8-DRB1*0301* haplotype is associated with lupus in northern European and north American populations. Two methods

have been used to determine whether the *C4* null allele association in lupus is causal or secondary to LD. One strategy involves the study of patients with lupus who do not bear *DR3* haplotypes for association with null alleles, while the second examines ethnically diverse populations in whom distinct extended haplotypes are observed. At present these data are inconsistent and require clarification by examining large lupus cohorts of defined ethnicity whilst accounting for patterns of LD. [20,31,36-38].

A role for another class III gene, tumour necrosis factor alpha (*TNF*), in SLE was suggested by McDevitt in 1988 following the observation that the lupus-prone New Zealand F1 mouse hybrid exhibits constitutively low *TNF* expression [39]. Recently the development of autoimmunity in patients treated with TNF-alpha antagonists has also stimulated interest in the possible role of TNF in SLE [40-42]. Case control studies have examined promoter single nucleotide polymorphisms (SNPs) and microsatellite polymorphisms at the *TNF* locus. Some studies have shown associations with certain *TNF* alleles [43-45]. However, the limited number of polymorphisms genotyped and the strong linkage disequilibrium between certain *TNF* alleles and the *B8-DR3* haplotype again restricts interpretation of these data.

In 2002, Graham et al [46] used a different strategy, that of family-based association to screen the human MHC region in lupus, albeit at low density. This analysis identified three microsatellite-inferred risk haplotypes in Caucasian lupus families: *DRB1*1501/DQB1*0602*, *DRB1*0301/DQB1*0201*, and *DRB1*0801/DQB1*0402*. Further analysis of ancestral recombinants could only delimit the disease associated region to 1 Mb of the MHC encompassing class II and class III.

Taking the above into account it is not surprising that our pooled analysis demonstrates predominant association with variants linked to *DR3* and *DR2*-bearing ancestral haplotypes in SLE (Figure 1). The strongest associations reside within the *DR3* haplotypes, *B8-DRB1*0301* and *B18-DRB1*0301*; specifically the majority of alleles map to the *B8-DRB1*0301* haplotype (*A1, B8, TNFb3, TNFa2, TNF-308A, TNFd1, C4A*Q0, DRB1*0301, DQA1*0501, DQB1*0201*). Half of the 26 significant positive associations lie on these two *DR3* haplotypes and display OR ranging from 1.5 to 2.5. Four of the remaining associations, *DR2*, *DR15, DRB1*1501*, and *DQB1*0602*, map to the *DR2/1501* haplotype, each with odds ratios of approximately 1.7. The association with another *DR2* subtype, *DRB1*1503* is seen in African Americans only, while the *DRB1*1602* allele is observed Mexican Mestizo, Thai, and Bulgarian populations. The observed *DRB1*0401* signal principally arises from Mexican Mestizo and Hispanic cohorts in whom this allele is uncommon (frequency ~1%). This association has not been well described and warrants further investigation. Two further class II alleles, *HLA-DQA1*0401* and *HLA-DQB1*0402*, reside on a *DR8* haplotype which is infrequent in Caucasian populations (frequency ~2%). The significance of the remaining associations with *HLA-B27* (in LD with *DRB1*0103, 0401, 0701* [47]) and the *TNF* microsatellites, *TNFb6, TNFa3, TNFa12* is unclear given the relatively small study numbers and wide confidence intervals.

This pooled analysis highlights the importance of polymorphism within *HLA-DR3*-containing haplotypes in lupus susceptibility. The remaining association

signals largely arise from the classical class II alleles *HLA-DRB1*, *HLA-DQA1* and *HLA-DQB1* and interestingly show evidence of population specificity.

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