Data S2

Type 1 Diabetes

Type 1 diabetes (T1D, MIM 222100) is a chronic autoimmune disease characterized by T cell-mediated destruction of pancreatic islet beta cells resulting in irreversible insulin deficiency and long-term dysfunction of several organs and tissues. Five main auto-antibodies, directed against islet antigens, are associated with disease: antibodies against insulin (IAA), glutamic acid decarboxylase-65 (GAD65), islet cell antigens (ICA), protein tyrosine phosphatase, ICA512 or IA2 (IA2) and zinc transporter ZnT8 (ZnT8A) [1]. The incidence of T1D varies greatly between different populations, ranging from 0.1/100 000/year in China to 36/100 000/year in Sardinia and Finland [2]. There is no doubt that the major genetic contribution to T1D susceptibility arises from the MHC [3]. This region, also designated IDDM1, is the only locus linked with T1D in every genome-wide screen to date. Several other genomic intervals have been linked to disease susceptibility, most notably the IDDM2 locus on chromosome 11 in the region of the insulin gene [4]. Approximately 50% of the total genetic contribution to T1D is attributable to the MHC [5] in comparison to only 15% in multiple sclerosis [6]. T1D susceptibility conferred by IDDM1 is complex and represents the combined effects of several susceptibility genes within the MHC [3]. Initial case-control studies of the MHC in T1D demonstrated associations with HLA-B serotypes [7]. These were subsequently shown to be due to linkage disequilibrium (LD) with the class II loci, HLA-DR and HLA-DQ [8]. It is now evident that the most important genes involved in T1D susceptibility at IDDM1
are HLA-DRB1, -DQA1, and -DQB1. To date most evidence supports a role for HLA-DQ as the major disease predisposing locus [9,10].

Most genetic studies in T1D have been undertaken in white Caucasian populations and consistently demonstrate disease predisposition with the haplotypes, DRB1*04-DQA1*0301-DQB1*0302 and DRB1*03-DQA1*0501-DQB1*0201 [8]. Heterozygosity for this combination of alleles confers the highest risk for T1D in several populations in a synergistic manner [8]. The formation of specific trans DQ dimers by transcomplementation between DQA1 and DQB1 alleles on homologous chromosomes (DQA1*0301/DQB1*0201 and DQA1*0501/DQB1*0302) may be responsible for the increase in heterozygote risk [3,11,12].

More than 90% of Caucasian individuals with T1D carry at least one of the two risk haplotypes: DRB1*04-DQA1*0301-DQB1*0302 and DRB1*03-DQA1*0501-DQB1*0201 compared with around 40% of the general population [13]. Therefore roughly 10% will carry neither haplotype. DR3/DR4 heterozygosity appears to influence age of onset in TID. This genotype occurs with greatest frequency in children who develop T1D before age 5 (50%) and least frequently in adults presenting with disease (20-40%), compared with a US population prevalence of 2.4% [14]. In addition, DR3/DR4 heterozygotes possess a 5% risk of developing T1D by age 15 [15]. DR4 (DRB1*0405-DQB1*0401) and DR9 (DRB1*0901-DQB1*0303) have shown association with T1D in Japanese and Korean populations [16]. The low frequency of the disease-associated DR3 and DR4 haplotypes may contribute to the reduced incidence of T1D in these non-white populations [8,17,18].
The nature of the HLA-DR association in T1D remains unclear. LD with HLA-DQ alleles may account for part of this association as DRB1*03 is in LD with DQA1*0501-DQB1*0201 and DRB1*04 is in LD with DQA1*0301-DQB1*0302 [3]. Other DRB1 alleles, in particular DRB1*04, may also modify the risk present at the DQ locus. DRB1*0401 and DRB1*0405 have been associated with increased disease risk in several populations independent of DQA1*0301-DQB1*0302, while DRB1*0403 and DRB1*0406 appear to confer protection from disease. The protective effect of DRB1*0403 appears to be dominant in that this allele was shown to overcome disease susceptibility in individuals carrying the highest risk genotype DQA1*0301-DQB1*0302 and DQA1*0501-DQB1*0201 [8]. The effect of predisposing DR3- and DR4-containing haplotypes is more consistent with a recessive model of inheritance [3]. The DQA1*0102-DQB1*0602 haplotype confers strong protection from T1D in Caucasian and Japanese populations [19-21]. Such protection dominates over the susceptibility encoded by the high-risk DQ alleles, but is not absolute. In contrast the DRB1*1501-DQA1*0102-DQB1*0602 haplotype is associated with an increased risk of other autoimmune diseases, such as multiple sclerosis and systemic lupus erythematosus. Different class II alleles, including DRB1*13-DQB1*0301, DRB1*11-DQB1*0301, DRB1*01-DQB1*0501, have shown evidence of protection in other populations [22]. Hence, determination of HLA-DQ (and -DR) status may prove beneficial in risk stratification for T1D in at-risk autoantibody positive individuals [23]. The presence or absence of various amino acid residues of the DRβ, DQα and DQβ peptide chains may be important in disease susceptibility by altering
the nature of the peptide binding groove. The absence of an aspartic acid residue at position 57 (Asp57) of the DQβ chain and the presence of an arginine residue at position 52 (Arg52) of the DQα peptide have been associated with susceptibility to disease. This hypothesis does not hold true for all T1D susceptibility alleles and the exact contribution of single or multiple amino acid residues to susceptibility remains to be determined [24]. A variety of studies have demonstrated association with HLA alleles and auto-antibody status in T1D; the most consistent findings are those of IAA, ICA, IA2 with DQ8 (DQB1*0302) [25,26] and GAD65 with DQ2 (DQB1*0201) [26-28]. It has yet to be established whether these antibodies are pathogenic or merely occur as a consequence of islet cell destruction.

More recently evidence is accumulating for the role of non-HLA loci within the MHC in susceptibility to T1D. A number of groups have shown that genes, including a polymorphism in ITPR3 (inositol triphosphate receptor 3), telomeric of class I may contribute to disease predisposition [29-32]. Polymorphisms of the MHC class I polypeptide-related sequence A, MICA, in the class III region, may lack an independent effect on genetic risk in T1D given that studies in different populations show inconsistent association of MICA alleles with disease and that associated alleles are often in LD with MHC class II risk haplotypes [33,34]. Promoter polymorphisms of another class III gene, TNF (tumour necrosis factor alpha), have been extensively studied in T1D [35-48]. Thus far, these associations have also been demonstrated to be secondary to LD with HLA class II alleles. Furthermore, DR-DQ independent effects in T1D have been shown with respect to age of
onset for *HLA-DPB1* [49,50], class I genes [51,52] and microsatellites within the class III region [53].

Overall, studies to date suggest that both *DR* and *DQ* genes are important in determining disease risk, but the effects of individual alleles may be modified by the haplotypes on which they are carried [8]. There appears to be a hierarchy of risk alleles from the strongly protective *DQB1*\(^*0602\) to the highly predisposing *DQB1*\(^*0302\). Such a spectrum of risk is also borne out by TDT analysis showing that each HLA-DR/HLA-DQ haplotype has its own individual disease risk which may result from transcomplementation and other haplotypic effects.

The main T1D association signals (29 out of 33) determined by this pooled analysis arise from *DR3*, *DR4* and *DR9*-containing haplotypes and concur with the published literature (Figure 1). The alleles of the *HLA-DR3* haplotypes that show positive association are: *A1, B8, MICA5.1, BfS1, C4A*\(^*Q0, DQB1*\(^*0201\) on AH8.1, B18, BfF1, DQB1*\(^*0201\) on AH18.2 and A33 on the less common AH58.1. Although *DRB1*\(^*0301\) and *BfF1* show the highest odds ratios (OR) in T1D, the wide confidence intervals suggest these results should be interpreted with caution. Of the four class III-associated alleles that reside on *DR3* haplotypes, *MICA5.1, BfS1, C4A*\(^*Q0\) are linked with AH8.1 and display OR between 1.4 and 2.8, less than the AH18.2 associated *BfF1* (OR 5.6). These data seem to corroborate previous reports of increased disease susceptibility conferred by AH18.2 compared with AH8.1 [32]. It is of interest to note that *DR3* (OR 3.8) and *DRB1*\(^*0301\) (OR 6.9) show a greater effect in comparison to *DQB1*\(^*0201\) (OR 2.9), even though these
two alleles are in strong LD. The opposite is seen with the DR4 haplotypic association, in relation to -DR and -DQ, where DQB1*0302 has an OR of 4.8 and DR4, DRB1*0401 and DRB1*0405 exhibit OR between 2 and 3.1. In keeping with the published literature the HLA-DR9 and HLA-DRB1*0901 associations observed in our pooled analysis arise from non-European cohorts only. The positively associated HLA-C alleles Cw1, Cw3 and Cw5, are all in LD with a range of ancestral haplotypes including the disease predisposing haplotypes, DR3 and DR4. The complement C4 allele, C4B5 maps to haplotypes containing DRB1*0405 and DRB1*1401, while DQB1*03032 may reside on DRB1*0701 and *0901 haplotypes. DRw53 or DR53 is the antigen encoded by one of the HLA-DRB genes, HLA-DRB4 and is found on DR4, -7 and -9 haplotypes.

DPB1*0201 maps to several disease-associated (DRB1*0401, 0405, 0301) and unrelated (01, 1601, 0701) haplotypes [54]. Studies demonstrate that HLA-DPB1 polymorphisms may alter the genetic effects of T1D-associated haplotypes, however, some of these specific HLA-DPB1 containing haplotypes are low frequency and their effect is small in Caucasian populations [54]. More recently, however, HLA-DPB1*0402 has been found to significantly protect against the development of anti-islet cell auto-antibodies in a high risk DR3-DQB1*0201/DR4-DQB1*0302 population [55]. Of the remaining associated alleles A9 (containing the splits A23 and the DR4-associated A24), B21, B41 and DMB*0104 do not map to specific ancestral haplotypes and may represent separate signals in T1D.
REFERENCES


leucocyte antigen-DR9-linked susceptibility to insulin-dependent diabetes mellitus. J Clin Endocrinol Metab 75: 1381-1385.


52. Valdes AM, Erlich HA, Noble JA (2005) Human leukocyte antigen class I B and C loci contribute to Type 1 Diabetes (T1D) susceptibility and age at T1D onset. Hum Immunol 66: 301-313.


55. Baschal EE, Aly TA, Babu SR, Fernando MS, Yu L, et al. (2007) HLA-DPB1*0402 protects against type 1A diabetes autoimmunity in the highest risk DR3-DQB1*0201/DR4-DQB1*0302 DAISY population. Diabetes 56: 2405-2409.