HLA-DP and coeliac disease: family and population studies

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Abstract

We investigated polymorphism of HLA-DP genes in three DR3 related diseases, confirming an association of coeliac disease with a Bgl II DPα polymorphism (a restriction fragment sized 3.5 kb present in 75% of patients compared to 34% of control subjects, p<0.001), and finding a weaker association with dermatitis herpetiformis (57% v 34%, p=0.01) and no association with insulin dependent diabetes mellitus. The association with coeliac disease was further investigated. Msp I DPβ polymorphism was studied in 52 healthy subjects and 59 patients: a 4.9 kb fragment was present in 51% of patients with coeliac disease compared to 11.5% of control subjects (p<0.001). Furthermore, nearly all subjects with the DPα 3.5 kb fragment also had the DPβ 4.9 kb fragment. However, disease frequency was still increased in the DPα 3.5 positive/DPβ 4.9 negative group. In seven families, each with at least two affected members, while the DPα 3.5 fragment was frequently present in patients it did not preferentially segregate with any particular HLA haplotype—for example, those associated with DR3 or DR7—and therefore is not part of an extended haplotype associated with coeliac disease. We therefore conclude that a gene in the HLA-DP region predisposes to coeliac disease independently of the HLA-DR/DQ regions.

Coeliac disease (CD) is characterised by damage to the intestinal mucosa with consequent malabsorption, which is reversible on withdrawal of the diet of the cereal protein gluten. A genetic component to this disease is well established by the high rate of concordance in monozygotic twins (70–100%) and an increased clustering in families (5–20% of first degree relatives have evidence of partial villous atrophy established by small bowel biopsy). Part of this genetic component in CD has been mapped to the major histocompatibility complex (MHC) located on the short arm of chromosome 6, with 95% of pairs of siblings with coeliac disease sharing one or more MHC haplotypes. The human MHC includes three subregions: class I (HLA-A, B, C), class II (proprotein Bf, complement C2 and C4, and 21 hydroxylase) and class II (HLA-DR, DQ, and DP), although many other genes have now been identified within this complex. The first associations between the MHC and CD to be found were with HLA-A1 and HLA-B8; subsequently closer associations were found with HLA-DR3, HLA-DR7, and HLA-DQw2. The population approach has been extended by Alper and colleagues who have shown tighter associations of CD with the extended haplotypes HLA-A1-B8-DR3-SCO1 and HLA-B44-DR7-FC31, and Hitman and Gorski et al have shown preferential allelic associations of CD based on serological specificities, restriction fragment length polymorphisms, and sequence variation of the HLA-DR and DQ genes. Furthermore, the extended HLA-DR3 haplotype is also found in other autoimmune diseases—for instance, insulin dependent diabetes mellitus and another gluten sensitive disorder, dermatitis herpetiformis.

Available evidence therefore indicates that the genetic component in CD is due to determinants at more than one locus. Based on monozygotic twin concordance data, the empirical sibling risks, and HLA gene sharing in siblings with CD, Rotter and Landaw have estimated that the described associations with HLA-A, B, C, and DR account for only 30% of the genetic component in CD. It is thus likely that other loci are involved in this multifactorial disease, and indeed recently HLA-DP genes have been implicated.

The purpose of this study was (i) to extend our original observations of the association of a Bgl II polymorphism of the DPα gene with CD to two other DR3 related diseases: insulin dependent diabetes mellitus and dermatitis herpetiformis; (ii) to examine segregation of the DPα polymorphism in families with CD; and (iii) to correlate polymorphisms of the DPα and DPβ genes in CD.

Patients and methods

All patients studied were unrelated white British, many of whom had been previously typed for HLA class I and class II antigens. Ninety nine control subjects were serially selected from staff at the London Hospital Medical College. None had a personal or family history of CD, dermatitis herpetiformis, or insulin dependent diabetes mellitus.

Patients with CD

(a) Population study. Sixty five patients were serially selected from clinics at either St Bartholomew's Hospital, London, or Queen Elizabeth Hospital for Children, Hackney. All had had an intestinal biopsy showing subtotal villous atrophy which was reversed after the withdrawal of gluten from the diet. All patients who also had dermatitis herpetiformis were excluded.

(b) Family study. Seven families with at least two members with CD (a total of 49 people, Fig 1), were recruited from the clinics at St James
Hospital, Dublin, Queen Elizabeth Hospital, Birmingham, the Luton and Dunstable Hospital, and St Bartholomew’s Hospital. Siblings and parents who did not have clinical symptoms suggesting CD were classified as healthy for this study. All but family 5 in this study were of Irish ancestry.

**PATIENTS WITH DERMATITIS HERPETIFORMIS**

Sixty-two patients were serially selected from the dermatology clinic at St Mary’s Hospital, London, who had spontaneous subepidermal blisters and deposits of IgA in the papillary dermis of unaffected skin. All cases of linear IgA disease were excluded.

**PATIENTS WITH INSULIN DEPENDENT DIABETES**

Seventy-eight patients with insulin dependent diabetes mellitus were recruited from the diabetic clinic at the London Hospital as previously described.16

**EXPERIMENTAL METHODS**

DNA was extracted from whole blood samples and studied by Southern blot hybridisation methods15 using the following probe-enzyme combinations: (i) a cDNA DPα probe (kindly donated by J Trowsdale, Imperial Cancer Research Fund, London) with the restriction enzyme Bgl II; (ii) a cDNA DPβ probe (from the Xth HLA International Workshop) with the restriction enzyme Msp I. Gene sequences were detected by autoradiography and sized by comparison with Hind III digested lambda phage and an internal laboratory standard.

**STATISTICS**

Statistical comparisons were made using χ² analysis and the method of Woolf to derive the relative risk and 95% confidence intervals.7

**Results**

**DPα POPULATION STUDY**

Using a DPα probe and the restriction enzyme Bgl II, two polymorphic fragments were identified, sized 3.5 and 2.2 kb. There was slight size variation of the 3.5-kb fragment (see Fig 2); a similar size variation was found in the corresponding subjects using a second enzyme, Taq I (data not shown). This size difference probably reflects the presence of a hypervariable region. Table I shows the frequency of the DPα 3.5-kb band in the three patient groups and in control subjects. There is a pronounced association with CD (75% compared to 34% in controls, p<0.001) and a weaker association with dermatitis herpetiformis (57% v 34%, p=0.01), but no association with insulin dependent diabetes mellitus. The presence of DPα 3.5 kb did not correlate with the presence of DR3 or DR7 in the control or patient populations.

**DPβ POPULATION STUDY**

Using a DPβ probe and the restriction enzyme Msp I the distribution of the four polymorphic fragments, sized 4.9, 3.0, 1.8, and 1.1 kb,16 was studied in patients with CD and control subjects (Fig 3). The principal findings (Table III) are an increase in frequency in CD of the 4.9-kb fragment (51% compared to 11.5% of controls; p<0.001) with a decrease in frequency of the DPβ 1.1-kb fragment (63% compared to 94% in controls; p=0.002).

The coinheritance of both the DPα 3.5 kb and DPβ 4.9-kb fragments was examined in 48 patients with CD and in 42 control subjects (Table IV); most of those with DPβ 4.9 kb also possessed DPα 3.5 (22/27, 81%), whereas there

**Table I**  HLA-DPα polymorphism (Bgl II) in patients with coeliac disease, dermatitis herpetiformis, and insulin dependent diabetes and control subjects

<table>
<thead>
<tr>
<th>Condition</th>
<th>DPα 3.5 kb (%), Relative risk</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control subjects</td>
<td>99/34</td>
<td>1.5</td>
</tr>
<tr>
<td>Coeliac disease</td>
<td>65/75*</td>
<td>2.9 to 11.8</td>
</tr>
<tr>
<td>Dermatitis herpetiformis</td>
<td>62/57*</td>
<td>1.3 to 4.8</td>
</tr>
<tr>
<td>Insulin dependent diabetes mellitus</td>
<td>78/44*</td>
<td>0.8 to 2.7</td>
</tr>
</tbody>
</table>

χ² analysis of disease v controls: *p<0.001, †p=0.01, ‡not significant.
was a significant number who possessed DPα 3.5 but not DPβ 4.9 kb (25/47, 53%), a combination which was increased in patients with CD (37%, 18/48 compared to 17%, 7/42 in control subjects, p=0.05).

**Discussion**

We have previously described an association of CD with the Bgl II DPα 3.5 kb polymorphism and from our current studies we conclude that there is a similar but weaker association in
dermatitis herpetiformis but not in insulin dependent diabetes. We now show that in CD (a) the DPβ polymorphism does not preferentially segregate with a particular HLA-DR/DQ phenotype in patients or controls, (b) there are associations with a DPβ polymorphism, and (c) there is an interrelationship between the DPα and DPβ polymorphisms.

In our extended CD (n=65) and control (n=99) panels we again observed the increased frequency of the 3.5 kb fragment in CD first detected by Niven et al. Simultaneously, Howell et al., using the restriction enzyme Xba I and a DPα probe, also reported an association of a 19 kb fragment with CD being present in 84% of 19 patients compared to 36% of 11 controls.

With the DPβ probe and the restriction enzyme Msp I, the 4.9 kb fragment was found in half our patients compared to just over 10% of controls. Similarly, Howell et al identified a 4 kb fragment with the enzyme Rsa I which was increased in CD patients, especially those with the DR3-DQw2 phenotype. Together with their limited DPα data they concluded that the DPα and DPβ restriction fragment length polymorphisms (RFLPs) associated with CD were in linkage disequilibrium with the corresponding DR and DQ polymorphisms, particularly DR3-DQw2. In our population data there was no correlation between the DPα RFLPs and any particular DR/DQ phenotype, nor did our family studies show segregation of DPα 3-5 with specific DR3 or DR7 haplotypes. Furthermore, we observed recombinants between the HLA-DR and DP regions in two of our families. This latter observation is to be expected as when the DP region was first defined (by the cellular primed lymphocyte typing method) no linkage disequilibrium between the DP and DQ/DR regions could be shown. We therefore conclude that the susceptibility gene associated with the DP region is different from those identified in the DR/DQ region.

Two groups have attempted to correlate DP typing with RFLP analysis. Of relevance to the present study, Hylidig-Nielsen had previously described the same DPα 3-5 kb and the DPβ Msp I polymorphisms that we studied and correlated those RFLPs with DP primed lymphocyte typing (PLT) in healthy Danish whites. Although there is a correlation of DP typing with DPα Bgl II and Msp I DPβ RFLPs, the latter method cannot be used for the accurate prediction of DP PLT and therefore differences in the distribution of PLT determined DP specificities between patients with CD and control subjects cannot be inferred.

Thus we have now shown at least three independent HLA-associated susceptibility alleles linked to CD, two located in the DR/DQ region and one in the DPβ region.
region^11^13^14^ and one located in the DP region. Whether the three alleles (or their products) interact to engender susceptibility to CD or whether they represent independent but additive susceptibility genes remains to be determined.

Preliminary evidence from de Koster and colleagues,^2^ that the DP antigen helps to present processed DR3 antigen to T helper cells provides a possible mechanism for interaction between the products of these two HLA subregions and hence a model for disease susceptibility. However, it should be noted that DP^a^ 3-5 kb is not necessary for CD within families: for instance, in family 6, of three siblings with CD only F1I-2 possesses DP^a^ 3-5 kb. Furthermore, in our limited family study two affected sibling pairs are non-HLA identical (family 1 and family 4); in previous reports only three out of 72 similar sibling pairs have been found to be HLA-non-identical. The fact that no one genetic marker has ever been found to be present in all patients with CD and that non-HLA identical sib pairs with CD each exist raises the possibility that different susceptibility alleles can be separately associated with CD and each can lead to disease, or that the susceptibility allele of a given haplotype can be inherited as part of a second haplotype within the family. Alternatively, susceptibility to CD may be dependent on the summated genetic load arising from alleles at a number of loci. As a result of such a threshold effect, combinations of alleles, differing between families and even individuals within families, may ultimately result in susceptibility to CD.

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