HLA-DP and coeliac disease: family and population studies

C Caffrey, G A Hitman, M J Niven, P G Cassell, P Kumar, L Fry, P Mackintosh, R Gallagher, C Feighery, D Weir, J A Sachs

Abstract

We investigated polymorphism of HLA-DP genes in three DR3 related diseases, confirming an association of coeliac disease with a Bgl II DP α 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 DP β 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 DP α 3.5 kb fragment also had the DP β 4.9 kb fragment. However, disease frequency was still increased in the DP α 3.5 positive/DP β 4.9 negative group. In seven families, each with at least two affected members, while the DP α 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(s) 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 from 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).¹ 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.²

The human MHC includes three subregions: class I (HLA-A, B, C), class III (properdin 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.^{3,5} The first associations between the MHC and CD to be found were with HLA-A1 and HLA-B8;^{6,7} subsequently closer associations were found with HLA-DR3, HLA-DR7, and HLA-DQw2.^{2,8–11} 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-SC01 and HLA-B44-DR7-FC31¹²; and Hitman *et al*¹³ and Gorski *et al*¹⁴ 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.¹⁵

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.¹ It is thus likely that other loci are involved in this multifactorial disease, and indeed recently HLA-DP genes have been implicated.^{15,16}

The purpose of this study was (i) to extend our original observations¹⁵ of the association of a Bgl II polymorphism of the DP α gene with CD to two other DR3 related diseases: insulin dependent diabetes mellitus and dermatitis herpetiformis; (ii) to examine segregation of the DP α polymorphism in families with CD; and (iii) to correlate polymorphisms of the DP α and DP β 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.¹³ 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

Medical Unit and
Department of
Immunology, The
London Hospital Medical
College, London
C Caffrey
G A Hitman
M J Niven
P G Cassell
J A Sachs

Department of
Gastroenterology, St
Bartholomew's Hospital,
London
P Kumar

Department of
Dermatology, St Mary's
Hospital, London
L Fry

Blood Transfusion
Service, Birmingham
P Mackintosh

Department of
Immunology and
Gastroenterology, St
James Hospital, Dublin,
Ireland
R Gallagher
C Feighery
D Weir

Correspondence to:
Dr G A Hitman, Medical Unit,
The London Hospital,
Whitechapel, London
E1 1BB.

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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.¹³

EXPERIMENTAL METHODS

DNA was extracted from whole blood samples and studied by Southern blot hybridisation methods¹³ 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.¹⁷

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 corres-

ponding 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.

DPα FAMILY STUDY

In six of the seven families there were at least two siblings with CD. Examining the two eldest affected siblings in each of these families and looking at the two generations in family 3, three siblings are HLA identical (families 2, 3 (FIII), and 5), two are haploidentical (families 3 (FII) and 6), and two are non-identical (families 1 and 4). Only four of the seven families (1, 3, 6, and 7) proved to be informative for segregation of HLA-DPα 3.5 kb with HLA serological typing. The DPα Bgl II restriction fragment length polymorphism did not consistently segregate with any particular haplotype (eg HLA-A1-B8-DR3, although present with DPα 3.5 on paternal haplotype in family 7 there are four other cases where it is in linkage with DPα 2.2 (Table II)). One definite recombination (FII-2 in family 7) between HLA-DP and HLA-DR was found in this study and also one probable recombination (FII-1 in family 4).

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,¹⁸ 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

	DPα 3.5 kb No positive (%)	Relative risk	95% confidence intervals
Control subjects	99 34		
Coeliac disease	65 75*	5.9	2.9 to 11.8
Dermatitis herpetiformis	62 57†	2.5	1.3 to 4.8
Insulin dependent diabetes mellitus	78 44‡	1.5	0.8 to 2.7

χ² analysis of disease *v* controls: **p*<0.001, †*p*=0.01, ‡not significant.

TABLE II Segregation of DPα 3.5 with class I (HLA-A and HLA-B) and class II (HLA-DR) antigens

	DPα 3.5	DPα 2.2
Family 1	A29-B44-DR7	A1-B8-DR3 A2-B51-DR2 A2-B62-DR4
Family 3	A11-B44-DR7	A2-B50-DR4 A1-B8-DR3 A1-B8-DR7
Family 6	A2-B12-DR2	A1-B8-DR3 A2-B13-DR7 A1-B37-DR6
Family 7	A1-B8-DR3 A2-B14-DR3	A29-B12-DR7 A1-B8-DR3

DPα 3.5 and DPα 2.2 are two DPα allelic fragments detected by Southern blot hybridisation methods and the restriction enzyme Bgl II.¹⁹ Family numbers refer to pedigrees as set out in Figure 1.

Figure 1: Pedigree analysis. RFLP=restriction fragment length polymorphism.

was a significant number who possessed DP α 3.5 but not DP β 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 DP α 3.5 kb polymorphism¹⁵ and from our current studies we conclude that there is a similar but weaker association in

Figure 2: Bgl II DP α autoradiogram. DNA was extracted from whole blood samples, digested with the restriction enzyme Bgl II, and studied by Southern blot hybridisation methods and a cDNA DP α probe. DP α 3.5 and 2.2 are indicated.

dermatitis herpetiformis but not in insulin dependent diabetes. We now show that in CD (a) the DP α polymorphism does not preferentially segregate with a particular HLA-DR/DQ phenotype in patients or controls, (b) there are associations with a DP β polymorphism, and (c) there is an interrelationship between the DP α and DP β 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.*¹⁵ Simultaneously, Howell *et al.*, using the restriction enzyme Xba I and a DP α 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.¹⁶

With the DP β probe and the restriction enzyme Msp I, the 4.9 kb fragment was found in

TABLE III HLA-DP β (Msp I) polymorphism in patients with coeliac disease and healthy controls (figures in parentheses are numbers)

DP β fragment (kb)	Positive (%)			
	Control subjects	Patients with coeliac disease	Relative risk	95% confidence intervals
4.9	11.5 (6/52)	51 (30/59)*	9.0	3.3 to 24.1
3.0	92 (33/36)	82 (43/52)†	0.5	0.1 to 1.8
1.8	27 (9/33)	33 (14/42)†	1.3	0.5 to 3.6
1.1	94 (48/51)	63 (33/52)‡	0.1	0.03 to 0.39

* $\chi^2=16.7$; $p<0.001$. †Not significant. ‡ $\chi^2=13.9$; $p=0.002$.

DP β 4.9 kb, 3.0 kb, 1.8 kb, and 1.1 kb are non-allelic fragments of DP β detected by Southern blot hybridisation methods and the restriction enzyme Msp I.¹⁸ Individuals in which the presence of a fragment was equivocal were excluded from the analysis; thus the number of individuals studied for each DP β fragment is not the same.

TABLE IV Correlation of DP α 3.5 kb and DP β 4.9 kb polymorphism in control subjects and patients with coeliac disease (percentages in parenthesis)

DP α 3.5, DP β 4.0	++	+-	-+	--
Control subjects (n=42)	3 (7)	7 (17)	2 (5)	30 (71)
Patients with coeliac disease (n=48)	19 (40)	18 (37)	3 (6)	8 (17)

++=DP α 3.5 positive DP β 4.9 positive; +- = DP α 3.5 positive DP β 4.9 negative; -+ = DP α 3.5 negative DP β 4.9 positive; -- = DP α 3.5 negative DP β 4.9 negative.

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.^{16,19} Together with their limited DP α data they concluded that the DP α and DP β 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 DP α RFLPs and any particular DR/DQ phenotype, nor did our family studies show segregation of DP α 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.²⁰ 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.^{18,21} Of relevance to the present study, Hyldig-Nielsen had previously described the same DP α 3.5 kb and the DP β Msp I polymorphisms that we studied and correlated those RFLPs with DP primed lymphocyte typing (PLT) in healthy Danish whites.¹⁸ Although there is a correlation of DP typing with DP α Bgl II and Msp I DP β 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

Figure 3: Msp I DP β autoradiogram. DNA was extracted from whole blood samples, digested with the restriction enzyme Msp I, and studied by Southern blot hybridisation methods and a cDNA DP β probe. Sizes of fragments are indicated.

region^{11 13 14} and one located in the DP region.¹⁵ 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,²² 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 α 3.5 kb is not necessary for CD within families: for instance, in family 6, of three siblings with CD only FII-2 possesses DP α 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.² 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 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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