HLA association with dermatitis herpetiformis is accounted for by a *cis* or *trans* associated DQ heterodimer

M A Hall, J S S Lanchbury, W J Bolsover, K I Welsh, P J Ciclitira

Abstract
HLA-DR, DQ, and DP restriction fragment genotyping was undertaken in 23 dermatitis herpetiformis patients and 53 healthy control subjects. HLA-DQw2 was present in 100% of patients with dermatitis herpetiformis (23 of 23) *versus* 40% of control subjects (21 of 53). Significant secondary associations occurred with HLA-DR3 (91% of patients *versus* 28% of control subjects) and DPw1 (39% of patients *versus* 11% of control subjects). Dermatitis herpetiformis and coeliac disease thus share an identical HLA class II association. It is likely that HLA class II genes directly influence the immune responses leading to mucosal damage in both diseases. The strongest candidate for disease susceptibility to dermatitis herpetiformis is DQw2. The HLA molecule most likely to be involved in coeliac disease is a specific DQα/DQβ heterodimer, encoded in *cis* arrangement in DR3 haplotypes or in *trans* arrangement in a DR5, 7 genotype. Our data on dermatitis herpetiformis patients fits this model perfectly. All these patients are capable of expressing this molecule, which may be responsible for the gluten sensitive enteropathy seen in a subgroup of patients with dermatitis herpetiformis and coeliac disease.

The common classic form of dermatitis herpetiformis is characterised by an itchy blistering skin eruption and granular IgA deposits at the dermoepidermal junction in an area not affected by the rash. Patients with classic dermatitis herpetiformis may exhibit gluten sensitive enteropathy, and the disease is associated with particular HLA class I and class II antigens. A less frequent form (seen in 10–15% of patients) is distinguished by linear IgA deposits at the dermoepidermal junction. This latter group have a normal or minimally abnormal intestinal mucosa and a normal or only slightly increased prevalence of particular HLA antigens. Because there is some controversy over whether this group is a subtype of dermatitis herpetiformis or a different disease altogether, these patients have been excluded from the study. Most patients with the classic form of dermatitis herpetiformis have no overt gastrointestinal symptoms. Gastrointestinal morphology is essentially identical to that seen in patients with ordinary coeliac disease, albeit less severe and more patchy.[1]

Previous serological studies point to the importance of a locus in the human major histocompatibility complex in determining susceptibility to dermatitis herpetiformis. HLA class I antigens A1 and B8 were initially shown to be associated with dermatitis herpetiformis.[2-4] Recent studies, however, suggest that the HLA class II antigens HLA-DR3 and DQw2 are more strongly associated with the disease.[5-8] These data indicate that the primary association of dermatitis herpetiformis is with the HLA class II region which includes loci encoding DR, DQ, and DP antigens. Any class I association is probably due to linkage disequilibrium. An identical association with the HLA-DR3 DQw2 haplotype has also been documented in coeliac disease.[9 10] A role for HLA in dermatitis herpetiformis is further suggested by comparisons of racial incidence *versus* HLA antigen frequency. This disease is very rare in Japan where the HLA B8 DR3 haplotype is almost absent in the normal population.[11]

Recent evidence strongly implicates the HLA-DP subregion in susceptibility to coeliac disease.[12-14] We and other investigators have shown that an extended HLA-DR3 haplotype including DPw1 is raised in coeliac disease.[15 16] Using primed lymphocyte typing it has been shown that this extended haplotype is also highly represented in dermatitis herpetiformis.[8]

Patients and methods
Twenty three unrelated white patients with dermatitis herpetiformis were selected on the basis of clinical and histological features of the disease and the presence of granular deposits of IgA in an area of the skin not affected by blistering. All patients had clinical or histological features, or both, of gluten sensitive enteropathy, ranging from total villous atrophy to a raised intraepithelial lymphocyte count. Controls were 53 healthy unrelated white subjects without any evidence of dermatitis herpetiformis. Patients and control subjects were typed for HLA-DR, DQ, and DP antigens using restriction fragment length polymorphism analysis.

GENOTYPING BY RESTRICTION FRAGMENT LENGTH POLYMORPHISM ANALYSIS
Genomic DNA was extracted from whole blood. Altogether 10 μg DNA were digested with 50 units of restriction enzymes TaqI and MspI according to the manufacturers instructions (Gibco BRL). Digested DNA was electro-phoresed on 0·6% agarose gels for 16 hours at 60 v (TaqI digested DNA) or 40 v (MspI digested DNA). Gels were Southern blotted onto Hybond-N according to manufacturers instructions (Amersham).

Probes used were a DRB1 SacI/Hind III

Molecular Immunogenetics Laboratory, United Medical and Dental Schools, Guy's Hospital, London
M A Hall
J S S Lanchbury
K I Welsh

Division of Pharmacology, United Medical and Dental Schools, St Thomas's Hospital, London
W J Bolsover
P J Ciclitira

Correspondence to:
Dr M A Hall, Molecular Immunogenetics Laboratory, 18th Floor, Guy's Tower, Guy's Hospital, London SE1 9RT.

Accepted for publication 16 June 1990

fragment of PII-β-4,[17] a DQB1 AvaI fragment of pII-β-1,[18] a DQA1 ApaI fragment of PII-β-5,[19] a DPB1 RsaI-HpaI fragment of p11β-7,[20] and a DPA1 EcoRI fragment of pDAα13b.[21] The probes were ^{32}P oligolabelled to specific activities of $3-5 \times 10^9$ counts per minute per μg DNA. Filters were prehybridised, hybridised, and washed at 65°C according to standard methods (Amersham) and autoradiographed on for one to seven days. TaqI filters were hybridised with DRB1, DQB1, DQA1, and DPA1 while MspI filters were hybridised with DPB1.

POLYMERASE CHAIN REACTION AMPLIFICATION OF GENOMIC DNA

Amplification of DNA was based on the procedure of Saiki et al.[22] For a standard reaction, 1 μg genomic DNA was subjected to 30 cycles of polymerase chain reaction using 2·5 units of Taq Polymerase (Perkin Elmer-Cetus) and DPB1 specific primers 259C and 257C at a 1 μmol/l concentration as previously described.[16] These primers span a 291 base pair segment encoding the hypervariable regions of the DPβ chain responsible for allelic variation. Each cycle consisted of 95°C denaturation (1·3 minutes), 55°C annealing (two minutes), and 72°C extension (three minutes), except for the initial denaturation step (95°C for 4·3 minutes) and the last extension step (72°C for 10 minutes).

ANALYSIS OF POLYMERASE CHAIN REACTION AMPLIFICATION PRODUCTS

Ten per cent of each polymerase chain reaction product was electrophoresed and blotted onto Hybond-N according to standard procedures

TABLE I Oligonucleotide probes defining DPw1 associated nucleotide sequences

Name	Sequence	Amino acids	DPB allele specificity
258C	5'TACGTGTACCAGGGACGGC	7–12	1,15,18
401D	5'GTCTGCATACCCTGTCC	74–78	1,3,7,8,9,10,12,14
402D	5'AGGAGTACGCGCGCTTC	34–38	1,11,13,15
849D	5'GAGCTGGTCGGGCCCAT	84–88	15,18

TABLE II HLA class II genotype and oligonucleotide data in 23 patients with dermatitis herpetiformis

Patient	HLA class II genotype			Oligonucleotide data			
	DR	DQ	DP	258C	401D	402D	849D
FB	3,7	2a2b	1,1	+	+	+	−
JB	3,7	2a2b	1,4	+	+	+	−
DC1	2,3	1b2a	1,4	+	+	+	−
JC1	3,3	2a2a	1,4	+	+	+	−
JC2	3,7	2a2b	4,3/6	−	+	−	−
DC2	2,3	1b2a	4,3/6	−	−	−	−
DC3	2,3	1a2a	1,2	+	+	+	−
MC	5,7	2b3b	4,4	−	−	−	−
EF	2,3	1b2a	1,4	+	+	+	−
EG	3,w8	2a3a	4,4	−	−	−	−
KH	3,w6	1a2a	2,5	−	−	−	−
DH	3,w6	1b2a	4,4	−	−	−	−
MJ	3,7	2a2b	1,5	+	+	+	−
PK	3,7	2a2b	3/6,M1.8	−	+	−	−
MK	3,w6	1a2a	4,3/6	−	+	−	−
SM	3,w6	1b2a	4,3/6	−	+	−	−
AR	3,7	2a2b	2,4	−	−	−	−
BR	2,3	1b2a	4,3/6	−	+	−	−
RS	5,7	2b3b	4,4	−	−	−	−
CS	2,3	1b2a	4,4	−	−	−	−
JS	3,4	2a3a	1,2	+	+	+	−
BT	3,3	2a2a	4,3/6	−	−	−	−
WT	1,3	1a2a	1,4	+	+	+	−

TABLE III Comparison of HLA, DR, DQ, and DP phenotype frequencies among dermatitis herpetiformis (DH) patients and control subjects. Fisher's p values, relative risks (RR), and aetiologial fractions (AF) are given below for statistically significant positive associations

Type	DH (n=23) % (No)	Control (n=53) % (No)
DR:		
1	−	26 (14)
2	26 (6)	30 (16)
3*	91 (21)	28 (15)
4	4 (1)	28 (15)
5	9 (2)	13 (7)
w6	17 (4)	24 (13)
7	35 (8)	28 (15)
w8	4 (1)	6 (3)
DQ:		
w1	48 (11)	66 (35)
w2†	100 (23)	40 (21)
w3	17 (4)	47 (25)
DP:		
w1‡	39 (9)	11 (6)
w2	17 (4)	25 (13)
w3/6	26 (6)	49 (26)
w4	70 (16)	81 (43)
w5	9 (2)	9 (5)
M1·8	4 (1)	6 (3)

* $p=1·9\times10^{-7}$, RR=22, AF=0·88. † $p=1·7\times10^{-7}$, RR=68, AF=1·00. ‡ p=0·008, RR=4·8, AF=0·32.

(Amersham). Duplicate filters with adjusted polymerase chain reaction products were each hybridised with one of a series of four sequence specific oligonucleotide probes. Probe specificities are given in Table I. Subjects who are positive with 258C, 401D, and 402D and negative with 849D have the DPB1 allele which is associated with the DPw1 specificity.

Oligonucleotide probes were end labelled, purified, hybridised, and washed according to published methods.[23] Hybridisation was carried out at 52°C (19mer oligonucleotide 258C) or 48°C (17mer oligonucleotides 401D, 402D, and 849D) for three hours followed by stringent washing at 56°C (258C) or 52°C (401D, 402D, and 849D) in 3·2M tetramethylammonium chloride (Fluka) 1% sodium dodecyl sulphate. Filters were autoradiographed for two to 12 hours at −70°C.

Statistical comparison of patient and control subject data was carried out using Fisher's exact test. Relative risks and aetiological fractions were calculated using the Woolf-Haldane method.

Results

DR and DQ genotypes were assigned according to the restriction fragment pattern seen from Taq1 digests of genomic DNA hybridised with DRB, DQB, and DQA cDNA probes.[24] DP genotypes were assigned according to the restriction pattern obtained using Msp1 and DPB1, and Taq1 and DPA1.[25] Genotypes assigned to the dermatitis herpetiformis patients are shown in Table II. M1.8 is a locally defined allele based on the presence of both 3·0 kb and 1·8 kb Msp1 DPB1 fragments. Comparisons of the distribution of DR, DQ, and DP genotypes in dermatitis herpetiformis patients and control subjects are shown in Table III, with the relative risks and aetiological fractions showing the strength of association. Since the intention of this study was to compare the associations of dermatitis herpetiformis with coeliac disease, the p values have not been corrected.
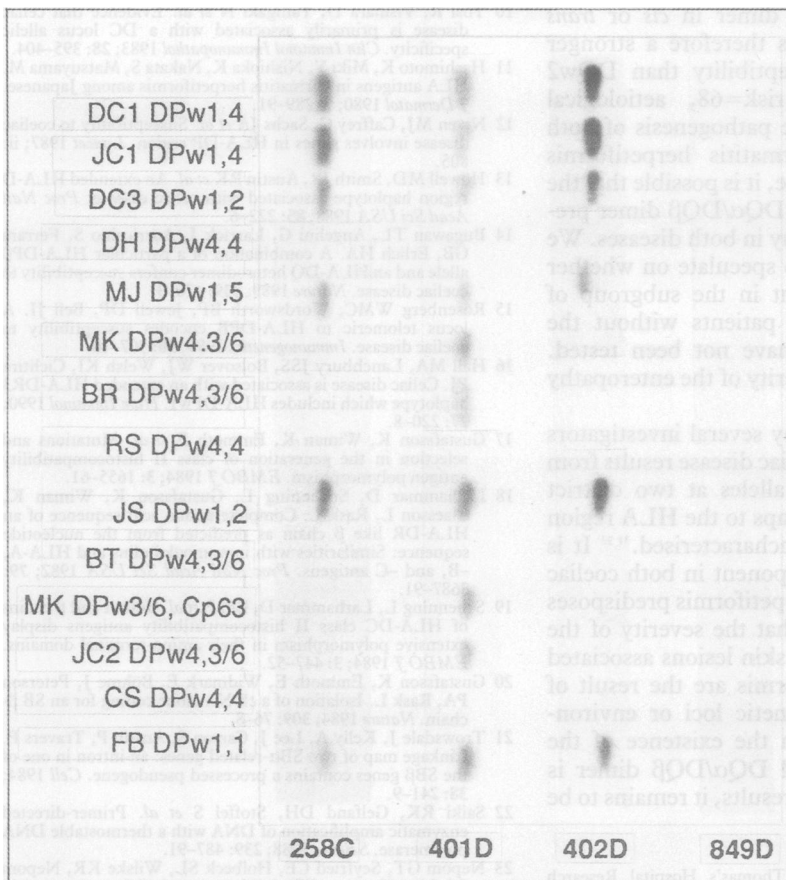
The strongest association is seen with HLA-

DC1 DPw1,4
JC1 DPw1,4
DC3 DPw1,2
DH DPw4,4
MJ DPw1,5
MK DPw4.3/6
BR DPw4,3/6
RS DPw4,4
JS DPw1,2
BT DPw4,3/6
MK DPw3/6, Cp63
JC2 DPw4,3/6
CS DPw4,4
FB DPw1,1

258C   401D   402D   849D

Southern blot analysis of polymerase chain reaction amplified DPB1 products from 14 DP genotyped dermatitis herpetiformis patients. Duplicate filters were hybridised with one of a series of four sequence specific oligonucleotide probes. Probe specificities are as follows: 258C is specific for DPB1,15 and 18; 401D is specific for DPB1,3,7,8,9,10,12, and 14; 402D is specific for DPB1,11,13, and 15 and 859D is specific for DPB15 and 18. Cp63 has been given the local allele designation M1.8.

DR3 and DQw2. Twenty one of 23 (91%) dermatitis herpetiformis patients are DR3 positive compared with 28% of control subjects (Fisher's exact test $p=1.9 \times 10^{-7}$, aetiological fraction=0.88). Twenty of the 21 DR3 positive patients have the Taq1 restriction fragment length polymorphism pattern associated with the A1 B8 DR3 haplotype.[26][24] The association with DQw2 is stronger – all 23 patients type as DQw2 compared with 40% of control subjects ($p=1.7 \times 10^{-7}$, aetiological fraction=1.00). Both these associations are comparable with those reported for coeliac disease. DP genotyping shows a significant increase in the frequency of DPw1 in dermatitis herpetiformis patients compared with control subjects (Table III). Nine of 23 dermatitis herpetiformis patients typed as DPw1 compared with 6 of 53 controls ($p=0.008$, aetiological fraction=0.32). We and others have previously shown a similar association with coeliac disease[27][15][16] and it has also been deduced for dermatitis herpetiformis (from primed lymphocyte typing data).[8] The Figure shows the hybridisation pattern of our four oligonucleotides in DP genotyped dermatitis herpetiformis patients. To confirm the restriction fragment length polymorphism determined DPw1 genotype, oligonucleotide probes targeting specific DPw1 associated DPB1 sequences were used (Table I). The DPw1 genotype assigned by restriction fragment length polymorphism analysis corresponds exactly with the pattern of

hybridisation of the oligonucleotides assigned to the DPB1 allele.

Independently, we have shown the DPB1 association at the molecular level, using both restriction fragment length polymorphisms and oligonucleotide analysis. Since we have shown that DR3 and DPw1 exist on an extended haplotype which is raised in coeliac disease, the results from this study suggest that the same extended haplotype is also highly represented in dermatitis herpetiformis.

Discussion

There is firm evidence for a shared genetic basis for the gluten sensitive enteropathy seen in coeliac disease and dermatitis herpetiformis. The strongest clue so far to understanding the shared pathogenesis is the mapping of susceptibility to the HLA class II region of the human major histocompatibility complex. This region contains genes coding for heterodimeric transmembrane glycoproteins which determine the magnitude and specificity of immune responses.[28] Coeliac disease and dermatitis herpetiformis share a similar immunopathology, namely T lymphocyte infiltration into the small intestinal intraepithelial layer and increased anti-gliadin antibody concentrations. The fact that the skin disease of many dermatitis herpetiformis patients can be controlled by a gluten free diet is further evidence of a relation. It is reasonable, therefore, to suppose that HLA class II genes directly influence specific immune responses leading to the characteristic mucosal damage. The first task in understanding the mechanistic link is to identify the HLA class II locus responsible. This is complicated by the extreme linkage disequilibrium shown by the A1 B8 DR3 DQw2 haplotype associated with both coeliac disease and dermatitis herpetiformis.

In this study we have examined molecular polymorphisms associated with DR, DQ, and DP loci among dermatitis herpetiformis patients and control subjects in order to isolate the primary susceptibility allele. Although dermatitis herpetiformis was significantly associated with DR3, DQw2, and DPw1, the strongest association is with DQw2, which must be considered the candidate allele for this disease. Since DR3, DPw1, and DQw2 are in linkage disequilibrium, the increase in DR3 and DPw1 can be explained by their existence on an extended haplotype which is highly represented in the disease population. This conclusion is further strengthened by recent reports that the HLA molecule most consistently associated with coeliac disease is a specific DQw2 DQα/β heterodimer encoded in cis arrangement on a DR3 haplotype.[29][30] Since the same DQα allele is also shared between DR3 and DR5 haplotypes, an identical transassociated DQα/DQβ dimer could be expressed by DR5, 7 subjects. Our data on dermatitis herpetiformis patients fits this model exactly. The two patients who do not type as DR3 are DR5, 7 and are capable of expressing the transassociated DQα/DQβ dimer. Thus 100% of dermatitis herpetiformis patients in contrast to 32% of control subjects ($p=5.2 \times 10^{-9}$, relative risk=98, aetiological fraction=1.00) have the

potential to express the dimer in *cis* or *trans* configuration, and this is therefore a stronger marker for disease susceptibility than DQw2 ($p = 1 \cdot 7 \times 10^{-7}$, relative risk=68, aetiological fraction=$1 \cdot 00$). Since the pathogenesis of both coeliac disease and dermatitis herpetiformis involves gluten intolerance, it is possible that the shared association with a DQα/DQβ dimer predisposes to the enteropathy in both diseases. We are not, however, able to speculate on whether this association is present in the subgroup of dermatitis herpetiformis patients without the enteropathy since they have not been tested. What determines the severity of the enteropathy is not known.

It has been proposed by several investigators that predisposition to coeliac disease results from recessive inheritance of alleles at two district genetic loci. One locus maps to the HLA region while the other one is uncharacterised.[31][32] It is likely that the HLA component in both coeliac disease and dermatitis herpetiformis predisposes to the enteropathy and that the severity of the mucosal damage and the skin lesions associated with dermatitis herpetiformis are the result of involvement of other genetic loci or environmental factors. Although the existence of the postulated *trans*associated DQα/DQβ dimer is strongly indicated by the results, it remains to be proved biochemically.

1 Scott BB, Losowsky MS. Patchiness and mucosal abnormality in coeliac disease (CD) and dermatitis herpetiformis (DH). *Gut* 1975; **16:** 393.

2 Katz SI, Falchuk ZM, Dahl MV, Rogentine GN, Strober W. HL-AB: a genetic link between dermatitis herpetiformis and gluten-sensitive enteropathy. *J Clin Invest* 1972; **51:** 2977–80.

3 White AG, Barnetson R St.C, Da Costa JAG, McClelland DBL. The incidence of HL-A antigens in dermatitis herpetiformis. *Br J Dermatol* 1973; **89:** 133–6.

4 Seah PP, Fry L, Kearney JW *et al*. A comparison of histocompatibility antigens in dermatitis herpetiformis and adult coeliac disease. *Br J Dermatol* 1976; **94:** 131–8.

5 Solheim BG, Ek J, Thune PO *et al*. HLA antigens in dermatitis herpetiformis and coeliac disease. *Tissue Antigens* 1976; **7:** 57–9.

6 Park MS, Terasaki PI, Ahmed AP, Zone J. The 90% incidence of HLA antigen (Te24) in dermatitis herpetiformis. *Tissue Antigens* 1983; **22:** 263–6.

7 Sachs JA, Awad J, McCloskey D *et al*. Different HLA associated gene combinations contribute to susceptibility for coeliac disease and dermatitis herpetiformis. *Gut* 1986; **27:** 515–20.

8 Hall RP, Sanders ME, Duquesnoy RJ, Katz SI, Shaw S. Alterations in HLA-DP and HLA-DQ antigen frequency in patients with dermatitis herpetiformis. *J Invest Dermatol* 1989; **93:** 501–5.

9 Demarchi M, Carbonara A, Ansaldi N *et al*. HLA-DR3 and DR7 in coeliac disease: immunogenetic and clinical aspects. *Gut* 1983; **24:** 706–12.

10 Tosi R, Vismara D, Tanigaki N *et al*. Evidence that celiac disease is primarily associated with a DC locus allelic specificity. *Clin Immunol Immunopathol* 1983; **28:** 395–404.

11 Hashimoto K, Miki Y, Nishioka K, Nakata S, Matsuyama M. HLA antigens in dermatitis herpetiformis among Japanese. *J Dermatol* 1980; **7:** 289–91.

12 Niven MJ, Caffrey C, Sachs JA *et al*. Susceptibility to coeliac disease involves genes in HLA-DP region. *Lancet* 1987; **ii:** 805.

13 Howell MD, Smith JR, Austin RK *et al*. An extended HLA-D region haplotype associated with celiac disease. *Proc Natl Acad Sci USA* 1988; **85:** 222–6.

14 Bugawan TL, Angelini G, Larrick J, Aurricchio S, Ferrara GB, Erlich HA. A combination of a particular HLA-DPβ allele and an HLA-DQ heterodimer confers susceptibility to coeliac disease. *Nature* 1989; **339:** 470–3.

15 Rosenberg WMC, Wordsworth BP, Jewell DP, Bell JI. A locus telomeric to HLA-DPB encodes susceptibility to coeliac disease. *Immunogenetics* 1989; **30:** 307–10.

16 Hall MA, Lanchbury JSS, Bolsover WJ, Welsh KI, Ciclitira PJ. Celiac disease is associated with an extended HLA-DR3 haplotype which includes HLA-DPw1. *Hum Immunol* 1990; **27:** 220–8.

17 Gustafsson K, Wiman K, Emmoth E *et al*. Mutations and selection in the generation of class II histocompatibility antigen polymorphism. *EMBO J* 1984; **3:** 1655–61.

18 Larhammar D, Schenning L, Gustafsson K, Wiman K, Claesson L, Rask L. Complete amino acid sequence of an HLA-DR like β chain as predicted from the nucleotide sequence: Similarities with immunoglobulins and HLA-A, –B, and –C antigens. *Proc Natl Acad Sci USA* 1982; **79:** 3687–91.

19 Schenning L, Larhammar D, Bill P *et al*. Both α and β chains of HLA-DC class II histocompatibility antigens display extensive polymorphism in their amino-terminal domains. *EMBO J* 1984; **3:** 447–52.

20 Gustafsson K, Emmoth E, Widmark E, Böhme J, Peterson PA, Rask L. Isolation of a cDNA clone coding for an SB β-chain. *Nature* 1984; **309:** 76–8.

21 Trowsdale J, Kelly A, Lee J, Carson S, Austin P, Travers P. Linkage map of two SBα-related genes: an intron in one of the SBβ genes contains a processed pseudogene. *Cell* 1984; **38:** 241–9.

22 Saiki RK, Gelfand DH, Stoffel S *et al*. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988; **239:** 487–91.

23 Nepom GT, Seyfried CE, Holbeck SL, Wilske KR, Nepom BS. Identification of HLA-Dw14 genes in DR4+ rheumatoid arthritis. *Lancet* 1986; **ii:** 1002–5.

24 Bidwell JL, Bidwell EA, Laundy GJ, Klouda PT, Bradley BA. Allogenotypes defined by short DQα and DQβ cDNA probes correlate with and define splits of HLA-DQ serological specificities. *Mol Immunol* 1987; **24:** 513–22.

25 Hyldig-Nielsen JJ, Morling N, Odum N *et al*. Restriction fragment length polymorphism of the HLA-DP subregion and correlations to HLA-DP phenotypes. *Proc Natl Acad Sci USA* 1987; **84:** 1644–8.

26 Bontrop RE, Tilanus M, Mikulski M, Van Eggermond M, Termijtelen A, Giphart M. Polymorphisms within the HLA-DR3 haplotypes. *Immunogenetics* 1986; **23:** 401–5.

27 Kagnoff MF, Harwood JI, Bugawan TL, Erlich HA. Structural analysis of the HLA-DR, –DQ, and –DP alleles on the celiac disease associated HLA-DR3 (DRw17) haplotype. *Proc Natl Acad Sci USA* 1989; **86;** 6274–8.

28 Kaufman JF, Auffray C, Korman AJ, Shackelford DA, Strominger J. The class II molecules of the human and murine major histocompatibility complex. *Cell* 1984; **36:** 1–3.

29 Roep BO, Bontrop RE, Pena AS, van Eggermond MCJA, van Rood JJ, Giphart MJ. An HLA-DQ alpha allele identified at DNA and protein level is strongly associated with celiac disease. *Hum Immunol* 1988; **23:** 271–9.

30 Sollid LM, Markussen G, Ek J, Gjerde H, Vartdal F, Thorsby E. Evidence for a primary association of celiac disease to a particular HLA-DQ α/β heterodimer. *J Exp Med* 1989; **169:** 345–50.

31 Pena AS, Mann DL, Hague NE. Genetic basis of gluten sensitive enteropathy. *Gastroenterology* 1978; **75:** 230–5.

32 Greenberg DA, Hodge SE, Rotter JI. Evidence for recessive and against dominant inheritance at the HLA – 'linked' locus in coeliac disease. *Am J Hum Genet* 1982; **34:** 263–77.