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HLA-DR phenotypes in Spanish coeliac children: their contribution to the understanding of the genetics of the disease

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SUMMARY The DR-locus controlled B-cell antigens were studied in 163 unrelated Spanish coeliac children and 68 families of this group, nine of them with more than one coeliac patient, to obtain more information about the association between these antigens and coeliac disease. The results show that the most common coeliac phenotypes are DR3/DR7, DR7/DR5, DR3/other DR, and DR3/DR3. The family study confirmed the segregation of the disease with the above mentioned phenotypes. In eight of the nine multiple case families, all coeliac children shared both HLA-DR antigens. These findings make it unlikely that a single dominant gene linked to HLA-DR controls the susceptibility to coeliac disease. The phenotypes in the patients were not distributed according to the Hardy-Weinberg equilibrium. Thus, a model based on one recessive susceptibility gene linked to HLA-DR is not probable either. The complexity of the genetics of coeliac disease and some of the features shared with the HLA-DR pattern in juvenile insulin-dependent diabetes are discussed.

Coeliac disease is known to have a hereditary character, but the mode of inheritance is still obscure. Extensive studies have shown an association between coeliac disease and HLA-B8,[1 2] and a still stronger one with HLA-DW3[3] and HLA-DR3.[4–6] It is also known that HLA-DR3 is not a specific marker for this disease. An association exists also with HLA-DR7.[7–11] Some authors have reported an excess of the HLA-DR genotypes DR3/DR7, DR3/DR3, and DR3/other DR.[8–11]

We have studied the DR locus-controlled B-cell antigens in Spanish coeliac children and their families to obtain more information about the association between these antigens and coeliac disease.

Methods

PATIENTS

A series of 163 unrelated Spanish coeliac children (99 girls and 64 boys; mean age eight years) and all first degree relatives of 68 of them were typed for the HLA-DR specificities. In all cases the diagnosis of coeliac disease was made in the Gastroenterology Unit of the La Paz Children's Hospital in Madrid according to the ESPGAN criteria.[12] Nine of the 68 families had two or more children affected by coeliac disease.

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CONTROLS

A group of 76 unrelated healthy Spanish volunteers served as the controls. All of them were typed for the known HLA-DR specificities.

HLA-DR LOCUS TYPING

Blood samples were taken in Madrid and flown to Amsterdam on the day of collection. HLA-DR typing was performed by the two colour fluorescence test[13] with a set of 60 platelet absorbed sera. The specificities HLA-DR 1, 2, 3, 4, 5, w6, 7, w8 and w9 could be recognised.

Results

POPULATION STUDY

The distribution of the HLA-DR locus specificities

in patients and controls is shown in Table 1. In the control group, the distribution of the HLA-DR antigens is in Hardy Weinberg equilibrium and the gene frequencies do not differ from those found in 181 healthy controls in Spain.[14] When the frequency of DRw6 was taken together with that given for DR6y in the above mentioned report, there was no divergence from the DRw6 frequency in our control group. A significant positive association between coeliac disease and HLA-DR3 and HLA-DR7 antigens is evident in Table 1, the relative risk for coeliac disease being 11·5 and 2·6 respectively. Table 2 shows that in this series by far the most common coeliac phenotype is DR3/DR7, followed by, in decreasing order, DR7/DR5, DR3/other DR, and DR3/undetermined. The notation undetermined is used for cases where no other DR-antigen was detected. The phenotypes DR3/DR7, DR7/DR5, and DR3/undetermined account for 68·7% of the patients studied versus 7·9% in the controls. Comparison between the frequencies of the phenotypes, in the coeliac disease and control group showed that these three phenotypes, namely DR3/undetermined, DR3/DR7, and DR7/DR5, are positively associated with coeliac disease. After correction for the number of comparisons, only the association with DR3/DR7 and DR3/undetermined proved to be statistically significant. It is interesting to note that 98% of the coeliac patients have one of the antigens DR3, DR7 or DR5.

FAMILY STUDY

The HLA-DR-genotype distribution of the 68 studied families are presented in Table 3.

In 31 of the 68 families, only one of the parents is heterozygous for HLA-DR3. In these families, 27 of the 31 coeliac children have the HLA-DR3 antigen, whereas in the group of healthy siblings the numbers of children with and without this antigen are distributed as would be expected according to the Mendelian segregation. A similar analysis for HLA-DR7 showed that in 27 families one of the parents is heterozygous for this antigen. Here, 22 of the 27 coeliac children have the HLA-DR7 antigen. In the healthy siblings the HLA-DR7 antigen shows a Mendelian distribution. Table 4 gives the segregation of the genotypes HLA-DR3/HLA-DR7 in 14 families with one parent heterozygous for HLA-DR3 and the other for HLA-DR7. The genotype HLA-DR3/HLA-DR7 is present in nine of the 14 coeliac children but in only six of their 29 healthy siblings, who show the expected distribution of the four possible HLA-DR genotypes. A similar analysis for genotype HLA-DR7/HLA-DR5 in five families where one of the parents is heterozygous for HLA-DR7 and the other for HLA-DR5 is also shown in Table 4. The genotype HLA-DR7/HLA-DR5 is present in four of the five coeliac children. Furthermore, in seven families where HLA-DR7 is present and HLA-DR3 is not, all of the coeliac children have the DR7/DR5 genotypes (Table 3). The nine families with more than one case were excluded from this segregation study.

MULTIPLE-CASE FAMILY STUDY

Among the nine families with more than one coeliac child, two have two identical pairs of twins concordant for coeliac disease who were excluded from the study. Table 3 shows that the remaining

Table 1 *HLA-DR antigen frequency distribution in 163 Spanish children with coeliac disease and 76 Spanish controls*

HLA-DR antigens	*Patients (N=163)* +	%	*Controls (N=76)* +	%	*Significance (p value)**	*Relative risk†*
DR1	4	2·45	15	19·74	$2 \cdot 10^{-4}$	0·11
DR2	3	1·84	22	28·95	$9 \cdot 10^{-9}$	0·05
DR3	116	71·17	13	17·10	$2 \cdot 10^{-13}$	11·54
DR4	20	12·26	15	19·73	ns	0·57
DR5	47	28·83	22	28·95	ns	0·99
DRw6	8	4·91	22	28·95	$6 \cdot 10^{-6}$	0·13
DR7	100	61·35	29	38·16	$8 \cdot 10^{-3}$	2·55
DRw8	2	1·23	3	3·95	ns	0·33
DRw9	1	0·62	0	0·00	ns	1·41

* Two-tailed p value corrected for number of antigens, calculated according to R A Fisher's chi-square, Yates corrected.
† According to Woolf, with Haldane's continuity correction.
ns = not significant.

Table 2 *HLA-DR phenotype distribution in coeliac patients and controls*

HLA-DR	*Patients (N=163)* +	%	*Controls (N=76)* +	%	*Significance* p*	*Relative risk†*
DR3/undetermined	20	12·27	0	0·00	$4 \cdot 10^{-2}$	21·8
DR3/DR5	12	7·36	4	5·25	ns	1·4
DR3/DR7	62	38·03	2	2·63	$2 \cdot 10^{-7}$	11·5
DR3/other	22	13·43	7	9·21	ns	1·5
DR5/undetermined	0	0·00	1	1·32	ns	0·2
DR5/DR7	30	18·40	4	5·56	ns	3·7
DR5/other	5	3·06	13	17·10	$4 \cdot 10^{-3}$	0·2
DR7/undetermined	2	1·23	4	5·26	ns	0·2
DR7/other	6	3·69	19	25·00	$2 \cdot 10^{-5}$	0·1
other/other	4	2·45	22	28·95	$4 \cdot 10^{-8}$	0·1

* Two-sided p value corrected for the number of comparisons (10) calculated according to chi-square, Yates corrected.
† According to Woolf, with Haldane's continuity correction.
ns = not significant.
The number of comparisons was 10 because of the nine phenotypes with either DR3 and/or DR5, and/or DR7 accounting for 97·5% among the patients. The other phenotypes (2·45%) were compound.

Table 3 *Segregation of HLA-DR antigens in 68 Spanish families with coeliac disease*

Family no	DR-genotype			
	Father	Mother	Coeliac children	Healthy children
1	7.w8	3.w6	7.3	7.w6
2	3.4	7.w6	3.w6	3.w6; 4.w6
3	7.1	3.3‡	7.3	1.3; 7.3
4	4.5	3.7	5.7	4.3; 5.3
5	7.2	3.w6	7.3†	2.w6; 7.w6; 2.3
				2.3; 2.w6; 7.3
6	3.1	7.7	3.7	3.7
7	4.2	5.5	4.5	
8	3.3‡	3.w6	3.3	3.w6
9	3.5	7.7	5.7	3.7; 3.7
10	3.5	7.5	5.7	5.5
11	7.1	3.4	7.3	1.3; 1.3; 1.4
12	3.3	7.1	3.7	3.7
13	3.5	3.4	5.3	3.3
14	3.4	3.4	3.3	4.4
15	3.2	7.2	3.7	2.2; 2.2
16	4.w6	5.2	4.5	w6.2; 4.5
17	7.5	5.5	7.5	5.5; 7.5; 7.5
18	7.4	3.4	4.3	3.7; 3.7
19	3.1	3.3‡	3.3	3.3
20	7.5	3.2	7.3; 7.3	7.2; 7.3
21	7.2	3.4	7.3	2.4; 2.3; 2.3
22	5.4	7.4	5.7; 5.7	
23	7.w6	5.1	7.5	7.5
24	3.7	3.5	7.3; 7.3; 7.3	7.5
25	3.2	1.w6	3.1*	3.1; 2.w6
26	3.7	7.4	3.7	7.4
27	3.3	3.4	3.3; 3.3	3.4; 3.4; 3.4
28	4.4	3.1	4.3	4.3
29	5.5	3.1	5.3	5.3
30	3.1	3.4	3.3†	3.3; 1.3
31	2.2	3.3	2.3; 2.3	2.3
32	3.5	7.7	3.7	5.7; 5.7; 3.7
				5.7
33	7.5	7.5	7.5	7.7; 7.5
34	3.5	7.w6	3.7	5.w6
35	3.2	7.7‡	3.7	
36	7.4	3.4	7.3	4.4; 7.3
37	3.w6	5.w8	3.5	
38	7.4	5.3	7.5; 7.3	
39	3.5	7.1	5.7	3.1
40	3.2	2.5	3.5	2.2
41	3.1	3.7	3.3	1.3
42	5.1	4.5	4.5	1.4; 5.5
43	3.5	7.7‡	7.3	5.7
44	w6.w6	w8.w6	w6.w6	w6.w6
45	3.3‡	3.2	3.3	3.3
46	7.5	w6.5	7.5	
47	7.2	3.w6	7.3	7.3; 2.3; 7.3
48	3.7	3.7	3.3	3.3
49	1.2	3.5	1.3	5.2
50	5.w6	7.w6	5.7	w6.7
51	3.2	4.4	3.4	3.4
52	3.7	3.3‡	3.3	3.3
53	3.2	7.w8	3.w8	3.w8
54	3.4	w6.5	3.w6	4.5
55	7.5	3.3	7.3	7.3; 7.3
56	3.3‡	7.w6	3.7	
57	3.w8	3.w6	3.3	w8.3
58	3.7	3.w10	7.3	7.w10
59	w6.1	3.5	w6.3	1.5
60	5.5‡	7.5	5.7	5.7
61	7.2	3.w6	7.3	7.w6
62	3.7	7.1	3.7	7.1
63	3.3‡	7.7‡	3.7	3.7
64	3.7	7.4	3.7	7.7
65	3.5	3.5	3.3	3.5
66	3.3	7.1	3.7; 3.7	
67	7.7‡	1.5	7.5	7.1; 7.1
68	3.3‡	7.7‡	3.7*	3.7

* Monozygotic twins concordant for coeliac disease. Only one individual is included in the Table.
† Monozygotic twins discordant for coeliac disease.
‡ Presumed homozygotes. DR-genotypes of parents are determined according to the four complete HLA-haplotypes in a family.

seven families comprise one family where both coeliac children are homozygous for DR3 (family 27) one where both coeliac children are heterozygous for DR3/DR2 (family 31) three families where all of the coeliac children are heterozygous for DR3/DR7 (nos 20, 24, 66), and one family where both coeliacs are heterozygous for DR7/DR5 (family 22). In one family (38) one of the coeliac children is heterozygous for DR7/DR5 and the other for DR3/DR7.

CALCULATION OF THE EXPECTED NUMBER OF DR PHENOTYPES

We calculated the expected numbers of DR phenotypes in our coeliac population from its gene frequencies as derived from the observed phenotypes according to the Hardy Weinberg equilibrium, assuming a recessive susceptibility gene, and compared the results with the observed phenotype frequencies (Table 5).

Discussion

Ten years have passed since the discovery of the association between coeliac disease and the HLA-B8 antigen.[1 2] Later it was shown that the disease is primarily associated with DW3,[3] and HLA-DR3.[4–7] Albert *et al*[7] were the first to observe that another antigen, namely DR7, was also associated with the disease, and these authors found an unexpectedly high number of heterozygous DR3/DR7. These findings were confirmed by DeMarchi *et al*.[8] The

Table 4 *Segregation analysis of genotypes HLA-DR3/HLA-DR7 and HLA-DR7/HLA-DR5 in Spanish coeliac families*

Coeliac children (n=14)*				*Healthy siblings (n=29)*			
DR3/DR7	DR3/−	DR7/−	−/−	DR3/DR7	DR3/−	DR7/−	−/−
9	3	2	0	6	10	3	10
Coeliac children† (n=5)				*Healthy siblings (n=6)*			
DR7/DR5	DR7/+	DR5/+	+/+	DR7/DR5	DR7/+	DR5/+	+/+
4	1	0	0	1	1	2	2

− = other antigen differing from HLA-DR3 or HLA-DR7.
+ = other antigen differing from HLA-DR7 or HLA-DR5.
* in 14 families with one parent heterozygous for HLA-DR3 and the other for HLA-DR7.
† in five families with one parent heterozygous for HLA-DR7 and the other for HLA-DR5.

present population study confirms the association of the disease with DR3 and DR7 and shows for the first time an increase in heterozygosity for DR7/DR5.

Our family study confirmed the segregation of the disease with HLA-DR3 and HLA-DR7 as well as with genotypes DR3/DR7 and DR7/DR5 (Table 4), and the present multiple case family study has shown that among six pairs and one family with three coeliacs, seven affected siblings are identical to the propositus with respect to the HLA-DR region. There is also one pair that does not share both antigens (Table 3, family 38), but in this family we can assume, on the basis of our present population and family data, that the different antigen inherited by the affected sibling is associated with the same susceptibility gene. In view of the segregation of coeliac disease in the families where the majority of the affected siblings share both HLA-DR antigens (Table 3), it seems very unlikely that a single dominant gene linked to HLA controls susceptibility to coeliac disease.

Table 5 *Expected and observed numbers of DR phenotypes in 163 coeliac patients*

DR phenotypes	*Gene frequencies**		
	Observed	*Expected*	χ^2
DR3/DR3	(11)† 20	28·37	2·47
DR3/undetermined	(9)		
DR3/DR5	12	19·61	2·95
DR3/DR7	62	42·55	8·89
DR3/other‡	22	17·10	1·40
DR5/undetermined	0	3·39	3·39
DR5/DR7	30	14·71	15·91
DR5/other	5	5·91	0·14
DR7/undetermined	2	15·96	12·21
DR7/other	6	12·83	3·63
other/other	4	2·58	0·78

χ^2=51·77; df=5; p<0·001.
DR3: [(2×20)+96] (2×163)=0·4172.
DR5: 47 (2×163)=0·1442.
DR7: [(2×2)+98] (2×163)=0·3129.
other DR: [(2×4)+33] (2×163)=0·1258.
undetermined = parents not typed; no other antigen detected.
df = degrees of freedom.
* blank alleles, whose gene frequency was <0·02, were omitted from the calculations.
† the frequency for DR3/DR3 was confirmed by family study.
‡ other antigen detected.

Greenberg *et al*[15] recently analysed and computed the published population HLA-DR data in coeliac disease. These authors used two models to compare the expected and observed phenotypes frequencies of DR3/DR7, one assuming that the susceptibility gene for coeliac disease linked to DR alleles is a dominant one and the other that it is a recessive gene. The findings contradict the dominant model and fit the recessive one. These authors correctly pointed out that their model should be applicable when the expected and observed numbers of homozygous DR3/DR3 and DR7/DR7 were similar. These data, however, were not available to them. Our present study (Table 5) shows a deficit of homozygotes for DR3/DR3, DR7/DR7, DR5/DR5, and an excess of DR3/DR7 and DR7/DR5, which makes a model based on one recessive susceptibility gene unlikely.

At this stage there seems to be no obvious explanation of these findings. Several possibilities should be investigated: (a) One possibility is that simple hereditary models with one susceptibility gene within the HLA-DR region, recessive or dominant, cannot explain the mode of inheritance of coeliac disease. In that case, more complicated models with more than one susceptibility gene linked to the HLA-DR region (two dominant genes, two recessive genes, one dominant and one recessive gene) should be investigated. As DeMarchi *et al*[8] have suggested, the susceptibility to coeliac disease might be determined by two genes within the HLA region, one in common with organ specific autoimmune diseases and in linkage disequilibrium with DR3, the other possibly specific for coeliac disease and associated with DR7. (b) It is also conceivable that our group of patients is not completely representative of the population of coeliacs, and that we failed to diagnose some of them because of the heterogeneity of the disease. The HLA-DR phenotypes of the coeliac patients might partially determine the mode of expression of the disease. For instance, it has been shown that when challenged with gluten coeliac children homozygous for HLA-DR3 display a more rapid

and stronger response both clinically and histologically, as shown by small intestine biopsy specimens.[16] Similarly, we have found that breast feeding delays the onset of symptoms in coeliac children with certain HLA-DR phenotypes.[17] On the other hand, it is known that coeliac disease can coexist with a number of autoimmune diseases, most of them related to HLA-DR3.[18–20] It seems possible that some of the HLA-DR3 homozygous patients are treated for some of these autoimmune diseases and are never diagnosed as having coeliac disease.

The absence of HLA-DR7 homozygotes in our series might be the result of a milder expression of the disease, because in less strongly affected patients a small intestinal biopsy is rarely performed. Another possible relevant point is that the present study, like most of those in which a relationship was found between coeliac disease and phenotype DR3/DR7,[8 10 11] was performed in children and, furthermore, from Southern Europe or Israel. It would be of interest to determine whether the association of coeliac disease with phenotypes DR3/DR7 and DR7/DR5 is a sign of the heterogeneity of the disease in relation to the patients' age or their geographic origin. A study of this kind done in adult French coeliac patients, however, also shows an association with phenotype HLA-DR3/HLA-DR7.[9] If heterogeneity of coeliac disease was actually related to the HLA-DR phenotype of the patients, each of the subgroups of coeliac disease might prove to have a specific mode of inheritance, as recently suggested by Rotter[21] for insulin dependent diabetes mellitus. (c) Finally, for a better understanding of the inheritance of coeliac disease we must not forget the important role that other factors – both hereditary (apart from the factors within the HLA-DR region) and environmental – can play in the expression of the disease. This is illustrated by the discrepancy between the degree of concordance for coeliac disease among identical twins (in our series 50%, in other series up to 70%[22]) and the 29% seen in coeliac children's siblings who share their HLA-DR phenotypes with the propositus and have coeliac disease (in our series eight out of 31). These findings indicate that there is around 28% (50–29) chance that hereditary factors not coded by HLA are involved in gluten sensitive enteropathy. Some of these factors have been explored elsewhere.[23] Greenberg and Rotter[24] have proposed that the mode of inheritance of coeliac disease can be explained by two recessive loci one within and one outside the HLA region. The discrepancy with respect to coeliac disease among identical twins shows clearly that a number of environmental factors – for example, age at first exposure to gluten, amount of gluten ingested, presence or absence of breast-feeding, intercurrent infections – play a role in the expression of the disease.

There can be no doubt from the present results that the complexity of the genetics of coeliac disease equals that found for juvenile insulin-dependent diabetes mellitus (IDDM).[25] In both diseases the number of homozygotes (DR3/DR3, DR4/DR4 for IDDM; DR3/DR3, DR7/DR7 for coeliac disease) is considerably smaller than the number of compound heterozygotes (DR3/DR4 for IDDM, DR3/DR7 for coeliac disease), and in both excess of haplotype-sharing by affected sib pairs has been found in multiple case families where all members were HLA-typed. Furthermore, in studies on HLA- and GLO-typed families with IDDM the susceptibility gene proved to be located closer to HLA-DR than to GLO.[26] The mode of inheritance of both diseases is still poorly understood. For coeliac disease, a model with one dominant susceptibility gene linked to the HLA-DR region seems very unlikely on the basis of the available data. The question whether one recessive susceptibility HLA-DR-linked gene can explain the hereditary pattern or a more complicated model is needed, deserves further clinical and experimental investigation. One important point concerns the possible heterogeneity of coeliac disease with respect to the HLA-DR phenotypes of the patients. We think that random HLA-DR and non-DR typing studies in coeliac patients differing as to age and ethnic and geographical origin will help to elucidate the still obscure aspects of the mode of inheritance of coeliac disease.

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