Genetic difference in HLA-DR phenotypes between coeliac disease and transitory gluten intolerance

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Abstract

Genetic differences in HLA phenotypes were studied in coeliac disease to investigate why some patients do not react with mucosal damage after gluten challenge. Forty five children with coeliac disease and 16 with transitory gluten intolerance were typed; 76 subjects served as controls. HLA phenotypes in children with coeliac disease had significantly higher proportions of DR3/X and DR5/7 than controls (48·8% *v* 11·8% and 26·7% *v* 5·3%). Children with transitory gluten intolerance had lower DR3/X (43·8%) than children with coeliac disease and there were no DR5/7 phenotypes.

Further analysis of similarly well defined cases might show whether genetic differences in the DR3/X and DR5/7 phenotypes can serve as a marker for the permanence of gluten intolerance.

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Keywords: HLA-DR phenotypes, coeliac disease, transitory gluten intolerance.

Coeliac disease has been recognised since 1888 when Samuel Gee first described the 'coeliac affection' as a disease entity with severe malabsorption and chronic diarrhoea combined with failure to thrive.[1] The typical hyperplastic villous atrophy of the proximal small intestine as a reaction to the toxic agent gluten[2] is considered to be mediated initially by an immunological reaction of the T lymphocytes of the mucosal lamina propria. These are predominantly of the CD4+ type.[3] Genetic control of this reaction is related to the major histocompatibility complex (MHC) region of chromosome 6, which encodes the MHC class I and class II molecules.[4 5] In this way coeliac disease affects genetically susceptible individuals. Susceptibility to coeliac disease has also been associated with HLA-A8,[6 7] HLA-DR,[8–11] and HLA-DQ[12 13] class antigens. In children with coeliac disease the permanence of the disease, according to the original European Society for Paediatric Gastroenterology and Nutrition (ESPGAN) criteria,[14] was demonstrated by gluten challenge after an initially flat mucosa, good recovery with a gluten-free diet, and morphological normalisation of the mucosa after following the diet for at least two years. Some patients did not react clinically and/or morphologically after gluten challenge according to these criteria, which led to the concept of transitory gluten intolerance.[15] It remains unclear whether genetic differences in the MHC class II molecules on the short arm of chromosome 6 could be responsible for this condition. Production of antigliadin antibodies of the IgG class and IgA class as well as antiendomysial antibodies have been shown to correlate with the immune response to gliadin.[16–19] In the mouse, two separate genetic loci on chromosomes 12 and 17 control the immune response to gliadin.[12] This study was performed to investigate whether there are genetic differences in the HLA-DR class antigens in children with coeliac disease and those with transitory gluten intolerance.

Patients and methods

PATIENTS

Two groups of patients, 20 boys and 41 girls, unrelated and aged 8 to 22 years were studied. Group I consisted of 21 patients in whom the diagnosis of coeliac disease was made by the typical clinical picture at presentation without initial biopsy, followed by a gluten-free diet for at least two years and good clinical recovery, then subsequent gluten challenge with histological reaction after having an intestinal biopsy. They were considered to have permanent coeliac disease. In the 40 subjects of group II, the diagnosis of coeliac disease was based on the original ESPGAN criteria. After the establishment of the diagnosis, shown by clinical presentation and hyperplastic villous atrophy in the first biopsy, the patients recovered taking a gluten-free diet for a minimum of two years and a second biopsy was performed before a prospective gluten challenge. All patients with coeliac disease are part of a large number of patients (n>450) being followed up in the hospital.

GLUTEN CHALLENGE

A third biopsy was performed after an initial standardised gluten challenge in all patients by giving 0·5 g/kg of body weight/day of gluten in the form of a powder added to the otherwise gluten-free diet for one month. Patients with a morphologically normal mucosa were changed to a normal diet. All patients with a histologically normal mucosa of group II (16/40) were consecutively changed to a normal diet. They remained on a normal diet for 5–15 years. Control biopsies were performed every two years during the

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Table 1 HLA-DR antigen frequency distribution in 45 Swiss children with coeliac disease and 529 Swiss controls

HLA-DR antigens	No (%) coeliac patients (n=45)	No (%) Swiss controls (n=529)	Significance (p values)*	Relative risk†
DR1	4 (8·9)	101 (19·1)	NS	0·46
DR2	2 (4·4)	150 (28·8)	<0·023	0·14
DR3	27 (60·0)	131 (27·8)	<0·001	4·05
DR4	4 (8·9)	148 (28·0)	NS	0·28
DR5+DR11	18 (39·9)	139 (26·3)	NS	1·88
DR6	4 (8·9)	108 (20·6)	NS	0·42
DR7	26 (57·7)	116 (21·9)	<0·0001	4·82
DR8	0	30 (5·7)	NS	0·18
DR9	1 (2·2)	11 (2·1)	NS	1·52
DR10	2 (4·4)	9 (1·7)	NS	3·15
DR11	15 (33·3)	118 (22·4)	NS	1·76

*Two tailed p value corrected for the number of antigens tested, calculated according to Fisher's χ^2 test with Yates's correction. †According to Woolf, with Haldane's continuity correction. NS=not significant.

Table 2 HLA-DR antigen frequency distribution in 16 Swiss children with transitory gluten intolerance and 529 Swiss controls

HLA-DR antigens	No (%) transitory gluten intolerance (n=16)	No (%) Swiss controls (n=529)	Significance (p values)*	Relative risk†
DR1	2 (12·5)	101 (19·1)	NS	0·73
DR2	1 (6·3)	150 (28·8)	NS	0·24
DR3	12 (75·0)	131 (27·8)	<0·001	8·42
DR4	2 (12·6)	148 (28·0)	NS	0·44
DR5+DR11	5 (31·3)	139 (26·3)	NS	1·34
DR6	2 (12·6)	108 (20·6)	NS	0·67
DR7	4 (25·1)	116 (21·9)	NS	1·28
DR8	2 (12·5)	30 (5·7)	NS	1·37
DR9	0	11 (2·1)	NS	1·66
DR10	0	9 (1·7)	NS	1·25
DR11	4 (25·0)	118 (22·4)	NS	1·76

*Two tailed p value corrected for the number of antigens tested, calculated according to Fisher's χ^2 test with Yates's correction. †According to Woolf, with Haldane's continuity correction. NS=not significant.

normal diet and no consequent relapses were demonstrated. They were considered to have transitory gluten intolerance.

CONTROLS

A group of 529 subjects were chosen as controls. They were MHC class typed because of evaluation as transplant donors or recipients or during the course of determination of paternity. Because they were not phenotyped, 76 controls from the study of Méarin *et al*[9] were taken for phenotype frequency of DR combination calculations.

HLA-DR LOCUS TYPING

Blood samples were subjected to HLA typing by standard lymphocytotoxicity techniques (National Institutes of Health). The specificities HLA-DR 1, 2, 3, 4, 5, 11(5), 6, 7, 8, 9, and 10 could be recognised.

Statistical analysis was calculated using the two tailed Student's *t* test corrected for the number of comparisons calculated according to Fisher's χ^2 test with Yates's correction. Relative risks were calculated according to Woolf, with Haldane's continuity correction.

Results

The distribution of HLA-DR locus specificities in patients with coeliac disease and controls is shown in table 1. In the group of 45 patients with coeliac disease (21 from group I, 24 from group II) there was a significant increase in the frequency of DR3 ($p<0·01$) and DR7 ($p<0·01$). In contrast DR2 was less frequent in patients with coeliac disease than in controls ($p<0·02$). In the remaining DR antigens no differences were found between the two groups. The relative risk for those patients demonstrating DR3 or DR7 was 4·05 and 4·82 respectively. In the group of patients with transitory gluten intolerance (n=16) DR3 was significantly more frequent ($p<0·01$), but not DR7 compared with controls (table 2). In the two groups of patients with coeliac disease and transitory gluten intolerance the frequency of DR3 was comparable: 60% *v* 75%, but DR7 was significantly less frequent in those with transitory gluten intolerance ($p<0·05$) (table 3). Phenotype distribution in patients with coeliac disease, those with transitory gluten intolerance and controls (taken from Méarin *et al*[9]) showed that patients with coeliac disease demonstrated a significantly higher distribution of DR3/DR7 ($p<0·01$) and DR5/DR7 ($p<0·05$) phenotype compared with the healthy controls. Patients with transitory gluten intolerance, showed also a higher distribution of DR3/DR7 phenotype compared with controls but surprisingly never DR5/DR7. This difference was significantly different ($p<0·05$) from patients with coeliac disease (table 3). All other phenotype distributions tested were no different from the controls, nor did they show any differences between the two patient groups. Compared with known populations of controls and patients with coeliac disease[20] in Norway, Spain, Argentina, Italy, and in our own study population the frequency of HLA-DR

Table 3 HLA-DR phenotype distribution in coeliac disease, transitory gluten intolerance, and controls

HLA-DR phenotype	No (%) controls (n=76)	Coeliac patients			Transitory gluten intolerance		
		No (%) (n=45)	Significance (p values)†	Relative risk‡	No (%) (n=16)	Significance (p values)†	Relative risk‡
DR1/undetermined	0	2 (4·4)	NS	8·8	2 (12·5)	NS	26·4
DR2/DR5+DR11	4 (5·3)	3 (6·7)	NS	1·3	3 (18·8)	NS	4·2
DR3/DR7	2 (2·6)	11 (24·4)	<0·01	9·9	2 (12·5)	NS	5·1
DR3/other	7 (9·2)	11 (24·4)	NS	3·1	5 (31·3)	NS	4·4
DR5/undetermined	1 (1·3)	0	NS	0·6	0	NS	1·5
DR5/DR7	4 (5·3)	12 (26·7)	<0·02	6·0	0	0·0165	0·5
DR5/other	13 (17·1)	3 (6·7)	NS	0·4	2 (12·5)	NS	0·8
DR7/undetermined	4 (5·3)	0	NS	0·2	0	NS	0·5
DR7/other	19 (25·0)	3 (6·7)	NS	0·2	2 (12·5)	NS	0·5
Other/other	22 (29·0)	13 (28·8)	<0·01	0·03	0	NS	0·07

NS=not significant. Undetermined=no other DR antigen detected (=probable DR homozygous).
*Taken from Mearin *et al*.[9] Own Swiss DR phenotypes were not determined.
†Two tailed p value corrected for the number of comparisons, calculated according to Fisher's χ^2 test with Yates's correction.
‡According to Woolf, with Haldane's continuity correction.

phenotypes DR3/X and DR5/5 was comparable in both but lowest for DR3/X. The patients with transitory gluten intolerance in our study group, however, showed lower DR3/X and no DR5/7 phenotypes.

Discussion

The definition of the permanence of coeliac disease has undergone some modifications and changes over the last 25 years. The first ESPGAN criteria of 1969, proposed at the Interlaken meeting, defined coeliac disease as (a) a structurally abnormal mucosa when taking a diet containing gluten, (b) clear improvement of villous structure when taking a gluten-free diet, and (c) deterioration of the mucosa during challenge.[14] The revision of these criteria in 1990 stated that the diagnosis of coeliac disease does not require further confirmation if the initial diagnosis is based firstly on the appearance of flat small intestinal mucosa with the histological features of hyperplastic villous atrophy and, secondly, on unequivocal and full clinical remission after withdrawal of gluten from the diet.[21] Exceptions to this rule are patients in whom the initial diagnosis was established before the age of 2 years and teenagers who tend to abandon the diet. In our study all patients have been challenged with gluten in a prospective manner regardless of the age of onset of coeliac disease. Sixteen subjects out of 61 did not react to gluten challenge and followed a normal diet afterwards for 5–15 years. They were considered to have suffered from transitory gluten intolerance. The number of patients with transitory gluten intolerance was thought to be not unusual because of the large number of proved coeliac patients (n>450) in the hospital. Genetic similarities with coeliac disease or normal controls would be of great help to classify them into either group, because the HLA-DQ genes of patients with coeliac disease are identical to those found among healthy subjects.[22] In studies in northern Europe close associations have been described with HLA class II alleles such as DR3 and DQ2 (DQA1*0501, DQB1*0201) that are found in 95% of coeliac patients from these regions.[12] In southern Europe, where the frequency of DR3 is lower among the control population, HLA class II associations are found significantly with DR3 and DR7.[23] The DR7 allele in these patients with coeliac disease is found in a heterozygous combination with either DR3 or DR5 alleles. Haplotype examinations show that the DR7 allele appears to be in linkage disequilibrium with the DQ alleles DQA1*0201 DQB1*0201, while the DR5 allele is found with the DQ alleles DQA1*0501 DQB1*0301. Individuals with the heterozygous combination of DR5/7 therefore carry the same combination of DQ alleles (DQA1*0501 DQB1*0201) as individuals positive for DR3/X. These DQ alleles are strongly associated with susceptibility of coeliac disease[20] and are expressed either in cis or transconfiguration suggesting that these molecules are responsible for the specific gliadin binding and thereby present the 'toxic' gliadin peptide to antigen specific T cells. In our group of patients, permanent coeliac disease was associated with DR3/X in 48·8% and DR5/7 in 26·7%. The DR5/7 association is dependent upon the geographic region and it was shown to have a frequency in our patients similar to southern Europe: 26·7% compared with 28% in Italy.[24] The frequency of DR5/7 is highest in Italy and lowest in Norway, probably due to the high frequency of the DR5 allele (49% in Italy, 13% in Norway, and 29% in Spain). The DR3/X frequency of 48·8% in our group of coeliac patients was lowest compared with other regions in Europe (Norway 95%, Spain 71%, Argentina 64%, Italy 60%),[18 24–26] but was still significantly higher than in all control groups in various regions of Europe (Norway 27%, Spain 17%, Argentina 15%, Italy 16%). In the group of patients with transitory gluten intolerance, DR3/X frequency of 43·8% was lower than in patients with coeliac disease regardless of geographic origin but considerably higher than in healthy controls in Italy.[24] As the DR alleles in our group of coeliac patients resemble those of the southern Europe group, transitory gluten intolerance might be thought to be associated with the southern European type of DR alleles. It is noticeable that our group of patients with transitory gluten intolerance carries more than twice the percentage of DR3/X alleles than that of controls from Italy. The striking difference from patients with coeliac disease or controls is shown by the absence of DR5/7 alleles. Despite the small patient numbers this might be relevant, because among the 16 patients with transitory gluten intolerance around 8% (or one patient) should have been found with the DR5/7 phenotype. Further analysis of similarly well defined cases might show whether genetic differences in the DR3/X and DR5/7 phenotypes can serve as a marker for transitory gluten intolerance.

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