Strategies for the identification and prevention of coeliac disease

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Phenotypic variance in childhood coeliac disease and the HLA-DQ/DR dose effect

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Abstract

Introduction: Coeliac disease (CD) is associated with HLA-DQ2 and-DQ8. The clinical picture is extremely variable and certain HLA-DQ/DR combinations have a higher relative risk (RR) for CD than others. Moreover, the HLA-DQ gene-dose effect has an impact on the strength of the gluten specific T-cell response and thus may correlate with clinical presentation and severity of CD.

Aim: To determine the correlation between HLA-DQ/DR based genotypes and the variation of phenotypes of the disease.

Methods: 113 non-related Caucasian children clinically diagnosed with CD during the period 1980-2003 with a known HLA type were included. Patients were divided into 4 categories according to amount of disease predisposing HLA-DQ2 or HLA-DQ8 molecules and the known relative risk (RR) of their HLA-DR/DQ type for CD: *high* (DR3DQ2 homozygous and DR3DQ2/DR7DQ2), *substantial* (DR3DQ2/DR5DQ7 and DR5DQ7/DR7DQ2), *moderate* (DR3DQ2-DR4DQ8 and DR3DQ2/DR*DQ*) and *low* (DR7DQ2/DR*DQ*, DR4DQ8- DR*DQ* and DR*DQ*- DR*DQ*). Clinical data and HLA-genotypes of these patients were compared.

Results: 113 children were diagnosed with CD at a mean age of 4.6 years and boys were significantly older diagnosed than girls (p=0.01). RR for having CD was highest for the *high* HLA-risk group (RR 8.1). Without the exception of more frequently abdominal distension and less non-gastro-intestinal symptoms in the *substantial* HLA-risk group, there were no significant differences in clinical characteristics or degree of severity of the small bowel histological findings between the children with different HLA-risk groups.

Conclusion: A correlation between disease severity and a double HLA-DQ2 gene dose was not observed.

Introduction

Coeliac disease (CD) is an inflammatory disorder caused by intolerance to the gluten proteins, which can be found in wheat, barley, rye, kamut and spelt, and characterized by a gluten-dependent enteropathy. In CD there is an abnormal immune response to specific gluten peptides, gliadins and glutenines, resulting in chronic inflammation and villous atrophy of the small intestine caused by an inflammatory T-cell response. (1) CD has a strong genetic component and most of the CD patients share major histocompatibility class II human leukocyte antigen (HLA) genes coded by the major histocompatibility region in the short arm of chromosome 6. More than 95% of CD patients share HLA-DQ2 (DQA1*0501-DQB1*0201), either in the *cis* (encoded by HLA-DR3-DQA1*0501-DQB1*0201) or in the *trans* configuration (encoded by HLA-DR5-DQA1*0501-DQB1*0301 / DR7- DQA1*0201-DQB1*0202) and most of the remainder have the HLA-DQ8 (encoded by HLA-DR4- DQA1*0301-DQB1*0302). (2;3) The mechanism by which HLA-DQ2 presents cereal peptides to intestinal T-cells is understood (4) and patients negative for both HLA-DQ2 and –DQ8 are very unlikely to develop CD.(5-7) Still, CD is a multigenic disorder, which means that the expression of these HLA-DQ2 or HLA-DQ8 molecules is necessary but not sufficient to cause development of the disease since approximately 30% of the Caucasian population holds the HLA-DQ2 haplotype and only 0.5% to 1% develops CD. Outside the HLA region there are several genomic areas related to CD, among others 5q31-33, containing cytokine encoding genes and genes associated with autoimmune or inflammatory conditions, 2q33 associated with the cytotoxic T lymphocyte antigen-4 (CTLA4 gene), Myosin IXB on 19p13 and IL2 and IL21 on 4q27 region, which plays a central role in immune response.(7-9) However, their contribution to the genetics of CD is relatively small and their mode of action in CD is still unclear.

The clinical picture of CD is variable.(10) In children, the classical presentation is characterized by chronic diarrhoea and malabsorption, abdominal distension and failure to thrive (the 'classical triad').(11) Other more subtle symptoms are, for example, chronic abdominal pain, lassitude or isolated short stature.(12) In CD, gastrointestinal symptoms may be absent or not prominent and patients may

present non-gastrointestinal symptoms of CD, e.g. lassitude and irritability.(13;14) In addition CD may be asymptomatic or accompanied by substantial morbidity, such as malignancies, osteoporosis, fertility problems and autoimmune disorders. CD is associated with a variety of diseases in some patients; mostly auto-immune diseases such as selective IgA-deficiency, dermatitis herpetiformis, Diabetes mellitus type I, auto-immune thyroiditis and Down syndrome.(10;14) It is not yet known what causes this clinical heterogeneity, but there is some evidence that genetic factors may be involved.(15-20)

The strong relationship between HLA genetic factors and CD is illustrated by the impact of the HLA-DQ2 gene dose on the chance of disease development: HLA-DQ2 homozygous individuals have an at least five times higher risk of disease development compared to HLA-DQ2 heterozygous individuals.(21;22) In addition, the gene dosage is determined by the amount of expressed HLA-DQ2/DQ8 in the small intestine, caused by the combination of the $\alpha\beta$ chains of the DQ2 (A1*0501, B1*0201 or A1*0201, B1*0202) and DQ8 (A1*0301, B1*0302) heterodimers. The gluten-specific T-cells respond more vigorously when gluten peptides are presented by antigen presenting cells homozygous for HLA-DQ2.(16; 23)

The aim of this study was to examine the possible relationship between the different clinical presentations of CD in children and their HLA-DRDQ haplotypes. We hypothesized that a gene dose effect accounts for variations in the clinical expression of the disease, because the more HLA-DQ-gluten complexes are formed, the stronger the T-cell reactivity will be.(23)

Materials and methods

Clinical data

We analysed the data from all the unrelated children with CD newly diagnosed at the Department of Paediatrics of Leiden University Medical Center (LUMC), between 1 January 1980 and 31 December 2003. CD was diagnosed according to the criteria of the European Society for Paediatric Gastroenterology Hepatology and Nutrition, based on the characteristic histological alterations for CD in small intestine biopsies.

(24) The age at diagnosis was defined as the age at the moment of the first diagnostic small bowel biopsy. The clinical data, among others sex, age, symptoms and growth status at diagnosis, CD-associated disorders, family history of CD and HLA-typing, were obtained from the medical files. All efforts were made to get missing data from patients who also attended other hospitals, for example by contacting their physicians.

HLA-typing was performed at the Tissue typing Laboratory of the Department of Immunohaematology and Blood Transfusion (LUMC) by means of the line probe method for HLA class II Low Resolution Typing.(25) After typing, the patients were grouped into four HLA genotypic categories according to the DR- and DQ-heterodimer combinations in single or in double dose and the consecutive amount of expression of disease predisposing HLA-DQ2 or HLA-DQ8 molecules in combination with their already known relative risk (RR).(23) These risk HLA-groups are: *high* (DR3DQ2 homozygous and DR3DQ2/DR7DQ2), *substantial* (DR3DQ2/DR5DQ7 and DR5DQ7/DR7DQ2), *moderate* (DR3DQ2/DR4DQ8 and DR3DQ2/DR*DQ*, different from DR3DQ2, DR4DQ8, DR5DQ7 or DR7DQ2) and *Low* (DR7DQ2/DR*DQ*, different from DR3DQ2, DR4DQ8, or DR5DQ7, and DR4DQ8/DR*DQ*, different from DR3DQ2, DR5DQ7, or DR7DQ2, and DR*DQ*/DR*DQ*, different from DR3DQ2, DR4DQ8, DR5DQ7, or DR7DQ2). Based on their HLA-risk group for CD, we compared the clinical data of these patients.

Statistical analysis

The strength of the association between the different HLA-risk groups and CD was calculated as relative risk (RR), which refers to the chance among children with CD of having the associated HLA-heterodimer in comparison to the general population. (26; 27). The correlation between the different HLA-risk groups and the clinical characteristics of the patients was analysed using the Kruskal-Wallis test for the numerical variables. The Mann-Whitney U test was used to compare the clinical characteristics of each HLA-risk group to the rest of the groups. These non-parametric tests are based on the sum of the ranks of the values in each of the groups; these should be comparable after allowing for differences in sample size.

The categorical variables were analysed using cross-tabulation and the Chi-square test, corrected with the Fisher's Exact test because of the small size of the groups. A p-value of <0.05 was accepted as statistically significant, but because of the small groups the total analysis was examined for trends.

Results

During 1980-2003, 185 children were diagnosed with CD at the LUMC. The HLA-typing was known in 149 of them (81 %). These children also included 27 with unrecognised CD found during a mass screening research project in apparently healthy children aged 2-4 years attending the Community Child Healthcare Centres in our area (13) and they were therefore analysed apart. Also, 5 siblings diagnosed with CD and 4 non-Caucasian CD patients were excluded to prevent bias in the outcome of the results. After these exclusions, our study group consisted of 113 unrelated Caucasian children. The mean age at diagnosis was 4.6 years (55.7 months, SD 42.4). The age at presentation of symptoms, known in 69 children, was 2.6 years (31.2 months; SD 30), with a mean lag time from the first symptoms to the diagnosis of 2.1 years (24.6 months; SD 31.6). The age at diagnosis was significantly higher among the boys (mean: 5.7 years, SD 3.7) in comparison to the girls (mean: 4.0 years, SD 3.3; p=0.01). There was no significant difference at the age at presentation among the boys (mean: 3.2 years) and the girls (mean: 2.3 years). The boy-girl ratio was 2 to 3 and both sexes were equally distributed over the different HLA risk groups. The distribution of the HLA-DR-DQ risk groups in the CD children with their RR for the disease is presented in table 1. As expected, the RR for CD in our population was highest in the DQ2 homozygous patients.

Table 2 shows the distribution of clinical characteristics in the different HLA-risk groups. There was no significant difference in the mean or median age at presentation of first symptoms (mean age: *high* 2.4yr*, substantial* 1.8yr*, moderate* 1.5yr *low* 1.5yr and median age: *high* 1.5yr*, substantial* 1.1yr*, moderate* 2.4yr, *low* 0.7yr) or at diagnosis of CD in the different HLA-risk groups (Table 2).

Table 1. Distribution of the HLA-DRDQ genotypes in Dutch children with coeliac disease (CD) in comparison with the Dutch general population

Risk group for CD	HLA-DRDQ genotype	CD (n=113) %	General Population (n=2307) %	Relative Risk (95% CI)
High	DR3 DQ2 / DR3 DQ2 DR3 DQ2 / DR7 DQ2	(45) 40	(114) 5	**8.1** (6.0-10.8)*
Substantial	DR3 DQ2 / DR5 DQ7 DR5 DQ7 / DR7 DQ2	(16) 14	(114) 5	**2.9** (1.8-4.7)*
Moderate	DR3 DQ2 / other** DR3 DQ2 / DR4 DQ8	(42) 37	(410) 18	**2.1** (1.6-2.7)*
Low	DR7 DQ2 / other† DR4 DQ8 / other‡ other / other**	(10) 9	(1669) 72	**0.1** (0.07-0.2)*

* *p < 0,05*
** *DR-DQ different from DR3DQ2, DR4DQ8, DR5DQ7 or DR7DQ2*
† *DR-DQ different from DR3DQ2, DR4DQ8 or DR5DQ7*
‡ *DR-DQ different from DR3DQ2, DR5DQ7 or DR7DQ2*

Table 2. Clinical characteristics of 113 children with coeliac disease in different HLA-risk groups

Symptoms	High (n=45) %	Substantial (n=16) %	Moderate (n=42) %	Low (n=10) %	Total %
Symptoms at presentation					
Chronic diarrhoea	49	44	41	50	45
Abdominal distension	42	75*	45	30	42
Failure to thrive‡	40	63	43	50	40
Abdominal pain	29	25	38	20	31
Non-GI symptoms†	51	25*	67	40	51
Asymptomatic	4	0	2	10	4
Mean age at diagnosis in years (SD)	5.0 (4.0)	3.8 (2.4)	4.6 (3.4)	4.5 (3.4)	4.6 (3.5)
Histology of small bowel biopsy					
STVA	71	88	64	50	69
PVA	29	12	36	50	31

HLA risk groups; high: DR3DQ2/DR3DQ2, DR3DQ2/DR7DQ2; substantial: DR3DQ2/DR5DQ7, DR5DQ7/ DR7DQ2; moderate: DR3DQ2/DR4 DQ8, DR3DQ2/other (see table I); low: DR7DQ2/other (see table I), DR4DQ8/other, other/other (see table I)
* *p<0.05*
‡ *defined as height and weight for age < p10*
† *among others lassitude, irritability, anaemia, mouth ulcerations and anorexia*

Moreover, the mean lag times from presentation to diagnosis were also similar between the different HLA-risk groups. Without the exceptions of more frequently abdominal distension and less non-gastrointestinal symptoms in the *substantial* HLA-risk group there were no significant differences in the clinical characteristics or in the degree of severity of the small bowel histological findings between the different HLA-risk groups. Figure 1 shows that the children in the *substantial* HLA risk group had significantly more frequent a small height for their age (< p10) than the children in the other groups.

Conditions associated with CD, such as diabetes mellitus type I, Down syndrome and selective IgA-deficiency, were significantly more frequent in children in the *moderate* HLA-risk group: 26% in comparison to 16%, none and 10 % in, respectively, the *high*, *substantial* and *low* HLA-risk groups (p=0.03).

The distribution of the HLA-DR-DQ risk groups in the 27 children with unrecognised CD found during mass screening in children aged 2-4 years is presented in table 3. None of the HLA-DR-DQ risk groups (*high*, *substantial, moderate* and *low)* were over-or-under represented in this group of apparently healthy children.

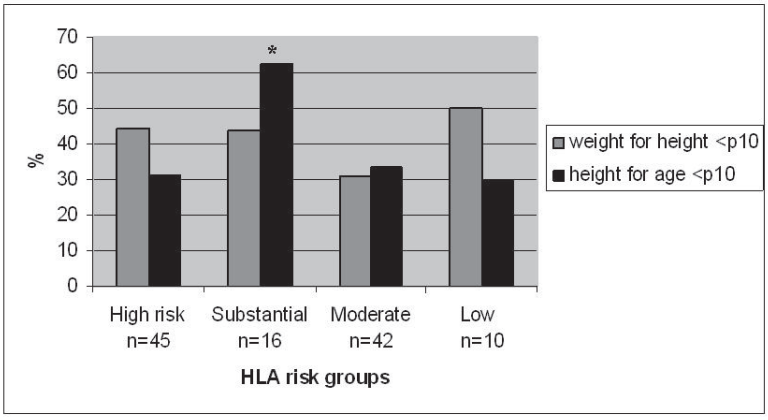

Figure 1. Growth status at diagnosis of 113 children with coeliac disease according to their HLA risk groups.

HLA risk groups: high = DR3DQ2 homozygous/ DR3DQ2-DR7DQ2; substantial = DR3DQ2-DR5DQ7/ DR5DQ7- DR7DQ2; moderate = DR3DQ2-otherDRDQ/ DR3DQ2-DR4DQ8; low = DR7DQ2- otherDRDQ/ DR4DQ8- otherDRDQ / otherDRDQ-otherDRDQ.

Discussion

Since the HLA-DQ2 gene dose has a strong quantitative effect on the magnitude of gluten-specific T-cell responses 'in-vitro'(23), we expected the most severe (classical) clinical presentation of CD within the children in the *high* HLA-risk group. However, our findings do not support the hypothesis of a correlation between disease severity at diagnosis and a double HLA-DQ2 gene dose (Table 2). Unexpectedly we found more abdominal distension and short stature, as well as fewer non-gastrointestinal symptoms among the children in the *substantial* HLA- risk group for CD (DR3DQ2/ DR5DQ7 and the DR5DQ7/ DR7DQ2). One question is whether our population is representative, since the population of CD children in a tertiary, university hospital referral centre for childhood CD, may be different from the CD population in general in the Netherlands. It is possible that cases difficult to diagnose may be seen more frequently at our hospital. On the other hand, our patient population was diagnosed with CD in the time period 1980- 2003, and it has been shown that the clinical presentation of CD has changed significantly between 1975-1990 and 1993-2000 (12), so in all probability our population presents with a mixture of the general clinical picture for CD in the Netherlands for the whole period. Moreover, the analysis of the distribution of the HLA-DR-DQ risk groups in the young children with CD found by mass screening shows that a double HLA-DQ2 gene dose is also presented in these children with subtle or no symptoms of CD (Table 3).

The relationship between the different HLA-DR and DQ haplotypes of children with CD and their clinical presentation has been investigated before. Some researchers have found a relationship between the gene dose effect and the clinical disease heterogeneity (15-19), but others have not found such an association.(20;28) It has been shown that CD children with the genotypes DR3(DQ2)-DR7(DQ2) and DR7(DQ2)-DR5(DQ7) had a significant difference in the increase in serum titters of IgA antigliadin antibodies after gluten challenge while the children homozygous for HLA-DR3(DQ2) did not.(15) Ploski *et al* (16) found a relationship between the late onset of CD and a double dose of DQB1*0201 (DR3DQ2 homozygous and DR3DQ2-DR7DQ2 patients), while other authors have found that a double dose of DQ2

predisposed for a more severe form of CD, more severe villous atrophy and a slower recovery of villous atrophy after following a gluten-free diet.(17-19; 28) In adults, it has been shown that homozygosity for HLA-DQ2 is associated with the development of refractory CD and enteropathy-associated T-cell lymphoma.(29) On the contrary, Greco *et al* (30) found no relationship between HLA-genotype and phenotype in CD children.

Table 3. Distribution of the HLA-DRDQ genotypes in 27 Dutch children 2-4 years with unrecognised coeliac disease (CD) identified during mass screening at the Well-Baby Clinics (13)

Risk group for CD	**HLA-DRDQ genotype**	**Children** n
High	DR3 DQ2 / DR3 DQ2 DR3 DQ2 / DR7 DQ2	9
Substantial	DR3 DQ2 / DR5 DQ7 DR5 DQ7 / DR7 DQ2	7
Moderate	DR3 DQ2 / other** DR3 DQ2 / DR4 DQ8	11
Low	DR7 DQ2 / other† DR4 DQ8 / other‡ other / other**	0

*** DR-DQ different from DR3DQ2, DR4DQ8, DR5DQ7 or DR7DQ2*
† DR-DQ different from DR3DQ2, DR4DQ8 or DR5DQ7
‡ DR-DQ different from DR3DQ2, DR5DQ7 or DR7DQ2

The differences in outcomes between the studies may be partially explained by the different classifications of the HLA-risk groups: double, single or no dose HLA-DQ2 of one allele versus different HLA-DR-DQ haplotypes as in our study. Also the description of the CD phenotypes differs in the different publications: from a general distribution in fully expressed disease versus mono-/oligosymptomatic (17) to an analysis of different symptoms or groups of symptoms as in our study. Another possibility is that some of the differences may be explained by the cohort-effect, which is likely to create differences based on different exposures to environmental factors that influence the appearance of disease, such as breast feeding, the amount of gluten in the diet and the prevalence of acute infectious diarrhoea.(29) A final

possible explanation could be that the different combinations of predisposing gene variants contribute to the observed differences in disease symptoms and age of onset and that additional non-HLA genetic factors may induce a more severe clinical expression in CD with these specific haplotypes. The recently reported strong risk for developing refractory CD in adults with the MYO9B gene illustrates this.(31) Further investigation, including gene expression analysis, will provide further insight into the genetic susceptibility for CD and its clinical expression.

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