Human Leukocyte Antigen–DQ2 Homozygosity and the Development of Refractory Celiac Disease and Enteropathy-Associated T-Cell Lymphoma

ABDULBAQI AL–TOMA,* MARIJE S. GOERRES,† JOS W. R. MEIJER,§ A. SALVADOR PEÑA,*,¶ J. BART A. CRUSIUS,¶ and CHRIS J. J. MULDER*

*Department of Gastroenterology, VU University Medical Center, Amsterdam, The Netherlands; †Department of Gastroenterology, and §Department of Pathology, Rijnstate Hospital, Arnhem, The Netherlands; and ¶Laboratory of Immunogenetics, Department of Pathology, VU University Medical Center, Amsterdam, The Netherlands

Background & Aims: **Celiac disease (CD) is a common gluten-sensitive enteropathy associated with human leukocyte antigen (HLA)-DQ2 and HLA-DQ8. The aim of this study was to determine if a particular HLA-DQ subtype predisposes to complications such as refractory CD with (RCD II) or without aberrant T cells (RCD I), and enteropathy-associated T-cell lymphomas (EATL).** ***Methods:*** **Molecular HLA-DQ typing was performed on 43 RCD I, 43 RCD II, and 30 EATL patients, and compared with age-matched groups of 121 patients with histologically defined uncomplicated CD and 183 healthy controls. All individuals were Dutch Caucasians and were at least 21 years of age.** ***Results:*** **HLA-DQ2 was present in 79% of RCD I, 97.7% of RCD II, and 96.6% of EATL patients. The differences were significant when compared with 28.9% in controls but not with 91.7% in uncomplicated CD. Homozygosity for HLA-DQ2 was observed in 25.5% of RCD I, 44.1% of RCD II, and 53.3% of EATL patients vs 20.7% of uncomplicated CD patients and 2.1% of controls. HLA-DQ8 was present in 10.7% of CD, 16.2% of RCD I, 9.3% of RCD II, and 6.6% of EATL patients vs 20.2% of controls.** ***Conclusions:*** **Homozygosity for HLA-DQ2 is associated with RCD II and EATL. Early identification of HLA-DQ2 homozygous CD patients may help to recognize CD patients at risk for developing these severe complications.**

Celiac disease (CD) is a common gluten-sensitive enteropathy affecting 1 in 150–300 individuals worldwide.[1,2] CD is associated strongly with the class II human leukocyte antigen (HLA)-DQ2 heterodimer encoded by the DQA1*0501 and DQB1*02 alleles. The DQ2 glycoprotein is present in 90%–95% of Caucasian CD patients.[3,4] The majority of DQ2-negative CD patients are positive for the haplotype DQA1*03-DQB1*0302 (HLA-DQ8).[5–7] A small number of CD patients lacking these heterodimers have either DQA1*05 or DQB1*02 alone.[8] CD-associated HLA-DQ molecules bind and present gluten peptides to antigen-specific T cells. These HLA-DQ–peptide complexes induce inflammatory T-cell responses in the small intestine with villous atrophy, crypt hyperplasia, and intraepithelial lymphocytosis.[4,9] HLA-DQ2 homozygous antigen-presenting cells induce higher T-cell proliferation and cytokine secretion than HLA-DQ2/non–DQ2 heterozygous antigen-presenting cells.[9] This may explain the strongly increased risk for disease development in HLA-DQ2 homozygous individuals.[10–13]

In a small percentage of patients serious complications develop. CD patients may be regarded as suffering from refractory CD (RCD) when symptoms persist or recur after a former good response despite strict adherence to a gluten-free diet. When normal expression of T-cell antigens and polyclonal T-cell receptor (TCR) gene rearrangement occur (RCD I) the prognosis is less dismal than when an aberrant clonal intraepithelial lymphocytes (IEL) population and/or loss of antigens on IELs is present (RCD II). These patients have a high risk for developing intestinal lymphoma.[14–17] Patients with refractory CD are at a greater risk for developing malignancy.[18] EATL has histologic and immunohistochemical features of large- or medium-size T-cell proliferation expressing a CD3+ CD8+/− and CD103+ phenotype. The majority of these lymphomas present as CD3+ CD8− CD30+ large-cell lymphoma, however, small-cell lymphomas, often CD3+ CD8+ CD30−, may occur.[19]

We have investigated whether a distinct HLA-DQ subgroup represents a risk factor for the development of refractory disease and the development of EATL.

Abbreviations used in this paper: CD, celiac disease; CI, confidence interval; HLA, human leukocyte antigen; IEL, intraepithelial lymphocytes; OR, odds ratio; RCD, refractory CD; TCR, T-cell receptor.

1542-3565/06/$32.00
doi:10.1016/j.cgh.2005.12.011

Table 1. Age and Sex Distribution of the Patients With Histologically Defined Uncomplicated CD (Marsh III), RCD I, RCD II, EATL, and Controls

	Mean age at diagnosis of CD, *y* (range)	Mean age at diagnosis of RCD I, RCD II, and EATL, *y* (range)	Male:female ratio
Uncomplicated CD (n = 121)	45.9 (22–75)	—	24:97
RCD I (n = 43)	47 (21–75)	49 (23–86)	12:31
RCD II (n = 43)	57 (40–69)	59 (47–88)	19:24
EATL (n = 30)	59 (46–69)	61.5 (52–79)	16:14
Controls (n = 183)	38.7 (24–89) at participation	—	85:98

Materials and Methods

Patients

Forty-three patients with RCD I (12 men, 31 women; mean age at diagnosis, 49 y; range, 23–86 y), 43 patients with RCD II (19 men, 24 women; mean age at diagnosis, 59 y; range, 47–88 y), and 30 patients with EATL (16 men, 14 women; mean age at diagnosis, 61.5 y; range, 52–79 y) were studied. Patients were referred to the Rijnstate Hospital or the VU University Medical Centre, both of which are tertiary referral centers for CD, and were recruited from all provinces in The Netherlands from 1992 to 2003. The patients with RCD I and II were followed-up over a mean period of 5 years (range, 2–12 y) for evidence of transition to a more severe state (ie, the transition from RCD I to RCD II and /or EATL, and from RCD II to EATL).

For comparison we used data on 121 unrelated and uncomplicated Dutch Caucasian CD patients (24 men, 97 women; mean age at diagnosis, 45.9 y; range, 22–75 y) selected by age to match the age groups under study. All these patients had villous atrophy (Marsh type III) on a normal gluten-containing diet and responded with histologic and clinical improvement to withdrawal of gluten from the diet.[20] Table 1 shows the demographic characteristics of these patients and the age at diagnosis of both CD and the complicated state (RCD and EATL).

A group of 183 unrelated healthy Dutch Caucasians (85 men, 98 women; mean age at participation, 38.7 y; range, 24–89), previously typed for HLA-DQ served as controls.[20]

In complicated CD, possible underlying diseases (except EATL) such as bacterial overgrowth, giardiasis, amyloidosis, intestinal lymphangiectasia, Whipple's disease, hypogammaglobulinemia, eosinophilic enteritis, and inflammatory bowel disease were excluded. In addition to endoscopic and histopathologic evaluation, all patients with complicated CD underwent clinical, laboratory, and radiologic assessment including intraepithelial lymphocyte phenotyping for signs of monoclonality; small-bowel radiograph and/or magnetic resonance imaging; serologic results for antigliadin, antiendomysium, and tissue transglutaminase levels; thyroid function tests; stool examination for giardia and other parasites; human immunodeficiency virus serology; and a dual-energy x-ray absorptiometry scan as part of a routine work-up.[1,17] When indicated, computed tomography scans of the abdomen, positron emission tomography, video capsule endoscopy, and/or double-balloon enteroscopy were performed.

Criteria for Diagnosis

The histology in the gluten-sensitive spectrum was categorized according to the modified Marsh criteria adapted by the working group of the 2001 United European Gastroenterology Week in Amsterdam.[21] The diagnosis of CD was confirmed by histologic examination with a documented histologic response to gluten withdrawal.[21] Patients with CD were considered to be refractory when symptoms of malabsorption owing to villous atrophy persisted or recurred after a former good response despite strict adherence to a gluten-free diet. The diagnosis of RCD was established as type I when no aberrant T cells were present in intestinal biopsy specimens and as type II when aberrant T cells were detected by immunophenotyping using flow-cytometric analysis or immunohistology of the intestinal mucosa.[17,21] In RCD I the IEL phenotype is normal with the expression of surface CD3, CD8, and TCR-β. In RCD II the IELs have normal cytologic features but they show an abnormal IEL phenotype with the expression of intracytoplasmic CD3ϵ, surface CD103, and the lack of classic surface T-cell markers such as CD4, CD8, and TCR-αβ.[18] The diagnosis of EATL was established according to the World Health Organization classification of tumors of hematopoietic and lymphoid tissues.[19,22,23] The immunohistochemical features of EATL are evidence of large- or medium-size T-cell proliferation expressing CD3+, CD8+/−, and CD103+. The majority are CD3+, CD8−, and CD30+ large-cell lymphomas; however, small-cell lymphomas often are CD3+, CD8+, and CD30−.[19]

Human Leukocyte Antigen–DQ Typing

Whole blood was obtained for typing of HLA-DQA1* and DQB1* alleles, performed with a combined single-stranded conformation polymorphism/heteroduplex method by a semiautomated electrophoresis and gel staining method on the Phastsystem (Amersham-Pharmacia-Biotech, Uppsala, Sweden).[20,24]

Statistical Analysis

Statistical data were analyzed by the Student *t* test, and results were considered statistically significant at a *P* value of less than .05. Odds ratios (ORs) and their 95% confidence intervals (CIs) were used to assess the significance of association between the dosage of haplotypes HLA-DQ2 and/or DQ8 and the risk for having RCD I, RCD II, and EATL.

Results

Table 1 shows the age and sex distribution of the patients with histologically defined uncomplicated CD (Marsh III), RCD I, RCD II, EATL, and controls.

Table 2. Distribution of Carriers of HLA-DQ2 and DQ8 Allelic Combinations in the Patients With Histologically Defined Uncomplicated CD (Marsh III), RCD I, RCD II, EATL, and Controls

	Controls (n = 183) (%)	Uncomplicated CD (n = 121) (%)	RCD I (n = 43) (%)	RCD II (n = 43) (%)	EATL (n = 30) (%)
DQ2/X	39 (21.3)	78 (64.5)	19 (44.1)	20 (45.4)	12 (40.0)
DQ2 homozygous	4 (2.1)	25 (20.7)	11 (25.5)	19 (44.1)	16 (53.3)
DQ2/DQ8	10 (5.5)	8 (6.6)	4 (9.3)	3 (6.9)	1 (3.3)
DQ2 positive	53 (28.9)	111 (91.7)	34 (79)	42 (97.7)	29 (96.6)
DQ8/X	25 (13.6)	4 (3.3)	3 (6.9)	1 (2.3)	1 (3.3)
DQ8 homozygous	2 (1.1)	1 (0.8)	0	0	0
DQ8 positive	37 (20.2)	13 (10.7)	7 (16.2)	4 (9.3)	2 (6.6)
Non-DQ2/non-DQ8	103 (56.3)	5 (4.1)	6 (14.0)	0	0
DQ2 or DQ8 positive	80 (43.7)	116 (95.9)	37 (86.0)	43 (100)	30 (100)

X = non-DQ2 and non-DQ8.

Table 2 shows the distribution of carriers of HLA-DQ2 and DQ8 allelic combinations in the patients with histologically defined uncomplicated CD, RCD I, RCD II, EATL, and controls. Forty-nine of 183 (26.8%) controls were found to be HLA-DQ2 heterozygous. Four (2.1%) controls were homozygous for DQ2. Thirty-seven controls (20.2%) carried the DQ8 haplotype; among them 2 individuals (1.1%) were homozygous for HLA-DQ8. Ten controls were positive for both HLA-DQ2 and DQ8 haplotypes. Altogether, 80 controls (43.7%) were positive for HLA-DQ2 and/or DQ8. Of note, in 103 controls (56.3%) HLA-DQ2 and DQ8 are absent.

In the patients with histologically defined uncomplicated CD (Marsh III), 111 of 121 (91.7%) patients carried at least 1 copy of the combination of genes DQA1*0501 and DQB1*02 (ie, were HLA-DQ2 positive), and 25 of 121 (20.7%) were DQ2 homozygous. Thirteen patients (10.7%) carried the haplotype HLA-DQ8 and 8 (6.6%) were positive for both HLA-DQ2 and DQ8. Therefore, 95.9% of patients with uncomplicated CD carried DQ2 and/or DQ8 markers.

In the RCD I group, 34 of 43 (79%) patients were DQ2 positive, and 11 of 43 patients (25.5%) were DQ2 homozygous. Seven patients (16.2%) carried the haplotype HLA-DQ8. Overall, 86% of RCD I patients carried HLA-DQ2 and/or DQ8. Six of 43 (14.0%) patients with RCD I had neither HLA-DQ2 nor DQ8, however, 3 of them had the allele DQB1*02.

In the RCD II group, 42 of 43 (97.7%) patients were HLA-DQ2 positive and 19 patients (44.1%) were HLA-DQ2 homozygous. Four patients (9.3%) carried the haplotype DQ8. Therefore, all RCD II patients carried HLA-DQ2 and/or DQ8.

In the EATL group, 29 of 30 (96.6%) patients were HLA-DQ2 positive and 16 (53.3%) were HLA-DQ2 homozygous. Two patients (6.6%) carried the haplotype DQ8. Therefore, all patients with EATL carried HLA-DQ2 and/or DQ8.

The mean age for CD diagnosis in the RCD II subgroup was 57 years (range, 40–69 y) and 59 years (range, 46–69 y) in the EATL group. Over a mean follow-up period of 5 years (range, 2–12 y), none of the RCD I patients had progressed to RCD II or EATL. Furthermore, of the 30 patients with EATL, the diagnosis was established in 5 without preceding known history of CD and/or RCD II, in other words, 25 patients progressed from RCD II to EATL.

Data on HLA-DQ2 homozygosity in RCD I, RCD II, and EATL and a comparison made with the histologic-defined uncomplicated CD patients (Marsh III) and controls are presented in Table 3. The difference in HLA-DQ2 homozygosity between RCD II (44.1%) and EATL (53.3%) was not significant ($P > .05$). RCD II and EATL patients have a statistically significant higher frequency of DQ2 homozygosity compared with uncomplicated CD (20.7%) ($P = .0046$; OR, 3.04; 95% CI, 1.44–6.41; and $P = .0003$; OR, 4.39; 95% CI, 1.91–10.08, respectively). No statistically significant differences were found in the carrier frequencies of HLA-DQ8 between uncomplicated CD, RCD I, RCD II, and EATL.

Table 3. HLA-DQ2 Homozygosity and the Risk for Having RCD I, RCD II, and EATL Compared With the Patients With Histologically Defined Uncomplicated CD (Marsh III) and Controls as Defined by OR and 95% CIs

Disease complication	ORs (95% CI) vs uncomplicated CD	*P* value vs uncomplicated CD	ORs (95% CI) vs controls	*P* value vs controls
RCD I	1.32 (.59–2.94)	.503	15.3 (4.8–48.5)	<.0001
RCD II	3.04 (1.44–6.41)	.0046	35.4 (11.5–107.6)	<.0001
EATL	4.39 (1.91–10.08)	.0003	51.1 (15.5–165.8)	<.0001

In relation to carriage of HLA-DQ2, the difference between RCD I and uncomplicated CD is significant ($P = .048$; OR, 3.0; 95% CI, 1.169–7.700), the difference between RCD II and RCD I does reach significance ($P = .0148$; OR, 11.118; 95% CI, 1.341–92.198), and the difference between EATL and RCD I is significant ($P = .0399$; OR, 7.676; 95% CI, .917–64.28). The difference in frequency of DQ2 homozygosity between RCD I (25.5%) and uncomplicated CD (20.7%) is not statistically significant ($P = .503$; OR, 1.32; 95% CI, .59–2.94).

Discussion

The relationship between HLA-DQ2, DQ8, and CD has become more clear in recent years. Through the activity of the enzyme tissue transglutaminase, glutamine residues in gluten are converted into glutamic acid. Subsequently, a multitude of gluten-derived peptides is generated that when bound to either HLA-DQ2 or DQ8 can induce T-cell responses in CD patients.[25,26] A particular glutamine and proline-rich 33-mer α-gliadin peptide that contains 6 different T-cell stimulatory sequences and is resistant to gastric and duodenal proteolysis might be the primary initiator of the inflammatory response to gluten.[27,28] In the large majority of patients, even in children with CD, inflammatory T-cell responses to other gluten peptides also are observed, implicating multiple gluten peptides in the disease process.[29]

Although reports by Zubillaga et al[7] and Congia et al[30] have shown that DQ2 homozygosity may predispose a person to an earlier onset and to more severe disease manifestations, Greco et al[31] found no correlation of clinical features of CD with different HLA-DR/DQ genotypes. Howell et al,[32] using frozen or paraffin-embedded biopsy tissue from 43 British EATL patients, found that EATL arises in individuals with the DQA1*0501, DQB1*02 CD-predisposing genotype, however, the patients were not homozygous for HLA-DQ2. In the present study, we found a highly significant correlation between HLA-DQ2 homozygosity and the development of serious complications of CD, in particular RCD II and EATL. We have no explanation for this discrepancy. However, Howell et al[32] found that 40% of EATL patients possessed the HLA-DRB1*03,04 genotype. Because 32.6% of the EATL patients carry allele HLA-DQB1*0302, which is in strong linkage disequilibrium with HLA-DRB1*04, a similar frequency of the haplotype DQA1*03-DQB1*0302 (HLA-DQ8) was to be expected in these Caucasian patients. The investigators did not comment on the unexpectedly low frequency (16.3%) of carriers of DQA1*03, suggesting some problems with genotyping these samples.

The link between HLA-DQ2 homozygosity and development of RCD II and CD-associated lymphoma of intraepithelial origin thus suggests that the strength of the gluten-specific T-cell response in the lamina propria directly or indirectly influences the likelihood of RCD II and lymphoma development. It has been reported earlier by Vader et al[9] that HLA-DQ2 homozygous antigen-presenting cells induce higher T-cell proliferation and cytokine secretion than HLA-DQ2/non–DQ2 heterozygous antigen-presenting cells. This may explain the strongly increased risk for disease development in HLA-DQ2 homozygous individuals.[10–13] This would indicate that the adherence to a gluten-free diet is particularly important for CD patients who are HLA-DQ2 homozygous.

Interestingly, none of the RCD II and EATL patients have been diagnosed with these complications at an age younger than 45, and only 1 of these patients was diagnosed with CD younger than the age of 45. These observations suggest that the specific tests such as those for CD3 cytoplasmic positive T-cells (immunohistology or T-cell flow cytometry) should be indicated in all patients with CD who are not responding to a gluten-free diet and who are older than age 45. Because the prognosis is very serious we propose to evaluate all "old-age celiacs" diagnosed with CD at an age older than 50.[18] The availability of a simple and reliable immunohistochemical method makes the distinction between CD and RCD feasible.[33] Although in this study we have not observed a transition from RCD I to RCD II, a prospective follow-up study with immunohistochemical techniques is indicated in HLA-DQ2 homozygous patients in particular. We strongly advise that for the time being at least full low-resolution HLA-DQA1 and DQB1 typing in CD should be performed. Techniques that recognize only the presence of HLA-DQ2 or DQ8 miss the few patients with CD who are non–HLA-DQ2 non–HLA-DQ8 and the possibility to diagnose HLA-DQ2 or DQ8 homozygotes.

Our observations require further confirmation in a larger group of patients and the set up of prospective studies. HLA-DQ typing is feasible and it may be an efficient test to recognize individuals at risk for these conditions with a poor prognosis, particularly now that some evidence has been given to support the hypothesis that autologous hematopoietic stem-cell transplantation can alter disease progression in severe autoimmune disease.[34,35]

References

1. Rostami K, Mulder CJ, Werre JM, et al. High prevalence of celiac disease in apparently healthy blood donors suggests a high prevalence of undiagnosed celiac disease in the Dutch population. Scand J Gastroenterol 1999;34:276–279.
2. Catassi C, Ratsch IM, Fabiani E, et al. Coeliac disease in the year 2000: exploring the iceberg. Lancet 1994;343:200–203.
3. Sollid LM, Markussen G, Ek J, et al. Evidence for a primary association of celiac disease to a particular HLA-DQ alpha/beta heterodimer. J Exp Med 1989;169:345–350.
4. Tighe MR, Hall MA, Barbado M, et al. HLA class II alleles associated with celiac disease susceptibility in a southern European population. Tissue Antigens 1992;40:90–97.
5. Spurkland A, Sollid LM, Polanco I, et al. HLA-DR and -DQ genotypes of celiac disease patients serologically typed to be non-DR3 or non-DR5/7. Hum Immunol 1992;35:188–192.
6. Polvi A, Arranz E, Fernandez-Arquero M, et al. HLA-DQ2-negative celiac disease in Finland and Spain. Hum Immunol 1998;59: 169–175.
7. Zubillaga P, Vidales MC, Zubillaga I, et al. HLA-DQA1 and HLA-DQB1 genetic markers and clinical presentation in celiac disease. J Pediatr Gastroenterol Nutr 2002;34:548–554.
8. Karell K, Louka AS, Moodie SJ, et al. HLA types in celiac disease patients not carrying the DQA1*05-DQB1*02 (DQ2) heterodimer: results from the European Genetics Cluster on Celiac Disease. Hum Immunol 2003;64:469–477.
9. Vader W, Stepniak D, Kooy Y, et al. The HLA-DQ2 gene dose effect in celiac disease is directly related to the magnitude and breadth of gluten-specific T cell responses. Proc Natl Acad Sci U S A 2003;100:12390–12395.
10. Mearin ML, Biemond I, Pena AS, et al. HLA-DR phenotypes in Spanish coeliac children: their contribution to the understanding of the genetics of the disease. Gut 1983;24:532–537.
11. Mearin ML, Bouquet J, Mourad N, et al. HLA-DR antigens and phenotypes in Dutch coeliac children and their families. Clin Genet 1985;27:45–50.
12. Ploski R, Ek J, Thorsby E, et al. On the HLA-DQ(alpha 1*0501, beta 1*0201)-associated susceptibility in celiac disease: a possible gene dosage effect of DQB1*0201. Tissue Antigens 1993; 41:173–177.
13. Louka AS, Nilsson S, Olsson M, et al. HLA in coeliac disease families: a novel test of risk modification by the 'other' haplotype when at least one DQA1*05-DQB1*02 haplotype is carried. Tissue Antigens 2002;60:147–154.
14. Biagi F, Corazza GR. Defining gluten refractory enteropathy. Eur J Gastroenterol Hepatol 2001;13:561–565.
15. Abdulkarim AS, Burgart LJ, See J, et al. Etiology of nonresponsive celiac disease: results of a systematic approach. Am J Gastroenterol 2002;97:2016–2021.
16. Wahab PJ, Meijer JW, Goerres MS, et al. Coeliac disease: changing views on gluten-sensitive enteropathy. Scand J Gastroenterol Suppl 2002;236:60–65.
17. Goerres MS, Meijer JW, Wahab PJ, et al. Azathioprine and prednisone combination therapy in refractory coeliac disease. Aliment Pharmacol Ther 2003;18:487–494.
18. Daum S, Cellier C, Mulder CJ. Refractory coeliac disease. Best Pract Res Clin Gastroenterol 2005;19:413–424.
19. Daum S, Ullrich R, Heise W, et al. Intestinal non-Hodgkin's lymphoma: a multicenter prospective clinical study from the German Study Group on Intestinal non-Hodgkin's Lymphoma. J Clin Oncol 2003;21:2740–2746.
20. Crusius JBA. The immunogenetics of chronic inflammatory and autoimmune disease. PhD thesis: Vrije Universiteit, Amsterdam, Netherlands, 2002.
21. When is a coeliac a coeliac? Report of a working group of the United European Gastroenterology Week in Amsterdam, 2001. Eur J Gastroenterol Hepatol 2001;13:1123–1128.
22. Isaacson PG. Intestinal lymphoma and enteropathy. J Pathol 1995;177:111–113.
23. Isaacson PG, Wright D, Ralfkiaer E, et al. Enteropathy-type T-cell lymphoma. World Health Organization classification of tumours: tumours of hematopoietic and lymphoid tissues. Lyon: 2001: 208–209.
24. Carrington M, Miller T, White M, et al. Typing of HLA-DQA1 and DQB1 using DNA single-strand conformation polymorphism. Hum Immunol 1992;33:208–212.
25. Dieterich W, Ehnis T, Bauer M, et al. Identification of tissue transglutaminase as the autoantigen of celiac disease. Nat Med 1997;3:797–801.
26. van de Wal Y, Kooy Y, van Veelen P, et al. Selective deamidation by tissue transglutaminase strongly enhances gliadin-specific T cell reactivity. J Immunol 1998;161:1585–1588.
27. Papadopoulos GK, Wijmenga C, Koning F. Interplay between genetics and the environment in the development of celiac disease: perspectives for a healthy life. J Clin Invest 2001;108: 1261–1266.
28. Shan L, Molberg O, Parrot I, et al. Structural basis for gluten intolerance in celiac sprue. Science 2002;297:2275–2279.
29. Vader W, Kooy Y, Van Veelen P, et al. The gluten response in children with celiac disease is directed toward multiple gliadin and glutenin peptides. Gastroenterology 2002;122:1729–1737.
30. Congia M, Cucca F, Frau F, et al. A gene dosage effect of the DQA1*0501/DQB1*0201 allelic combination influences the clinical heterogeneity of celiac disease. Hum Immunol 1994;40: 138–142.
31. Greco L, Percopo S, Clot F, et al. Lack of correlation between genotype and phenotype in celiac disease. J Pediatr Gastroenterol Nutr 1998;26:286–290.
32. Howell WM, Leung ST, Jones DB, et al. HLA-DRB, -DQA, and -DQB polymorphism in celiac disease and enteropathy-associated T-cell lymphoma. Common features and additional risk factors for malignancy. Hum Immunol 1995;43:29–37.
33. Patey-Mariaud De Serre N, Cellier C, Jabri B, et al. Distinction between coeliac disease and refractory sprue: a simple immunohistochemical method. Histopathology 2000;37:70–77.
34. Gratwohl A, Passweg J, Bocelli-Tyndall C, et al. Autologous hematopoietic stem cell transplantation for autoimmune diseases. Bone Marrow Transplant 2005;35:869–879.
35. Wulffraat NM, Vastert B, Tyndall A. Treatment of refractory autoimmune diseases with autologous stem cell transplantation: focus on juvenile idiopathic arthritis. Bone Marrow Transplant 2005;35(Suppl 1):S27–S29.

Address requests for reprints to: Professor C. J. J. Mulder, Department of Gastroenterology, VU University Medical Centre, PO Box 7057, 1005 MB Amsterdam, The Netherlands. e-mail: cjmulder@vumc.nl; fax: (31) 20-4440554.

The authors thank C. M. Gundy, PhD, Department of Clinical Epidemiology and Biostatistics, VU University Medical Center, for his kind assistance with the statistical analysis of the data.