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HLA Types in Celiac Disease Patients not Carrying the *DQA1*05-DQB1*02* (DQ2) Heterodimer: Results From the European Genetics Cluster on Celiac Disease

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ABSTRACT: Genetic susceptibility to celiac disease is strongly associated with *HLA-DQA1*05-DQB1*02* (DQ2) and *HLA-DQA1*03-DQB1*0302* (DQ8). Study of the HLA associations in patients not carrying these heterodimers has been limited by the rarity of such patients. This European collaboration has provided a unique opportunity to study a large series of such patients. From 1008 European coeliacs, 61 were identified who neither carry the DQ2 nor DQ8 heterodimers. Fifty seven of these encoded half of the DQ2 heterodimer. The remaining 4 patients had a variety of clinical presentations. Three of them carried the *DQA1*01-DQB*05* haplotype as did 20/61 of those carrying neither DQ2 nor DQ8. This may implicate a role of the *DQA1*01-DQB*05* haplotype. None of these four patients carried the *DQB1*06* allele that has previously been reported in this sub-group of patients. Of the 16 DQ2 heterodimer negative patients without *DRB1*04* or *DRB1*07* haplotypes, it was inferred that none encoded the previously implicated *DRB4* gene as none had a *DRB1*09* haplotype. These results underline the primary importance of HLA-DQ alleles in susceptibility to celiac disease, and the extreme rarity of celiac patients carrying neither the DQ2 or DQ8 heterodimers nor one half of the DQ2 heterodimer alone. *Human Immunology 64, 469–477 (2003).* © American Society for Histocompatibility and Immunogenetics, 2003. Published by Elsevier Science Inc.

KEYWORDS: celiac disease; HLA; DQ2; DQ8; genetics; genotypes

INTRODUCTION

Celiac disease is a strongly heritable disease with concordance between monozygotic twins of at least 75% compared to 11% in dizygotic twins [1]. A significant proportion of the genetic predisposition comes from human leukocyte antigen (HLA) linked genes, estimated to account for up to 40% of the genetic load [2]. Indeed, in European Caucasian populations, more than 90% of celiacs carry *HLA-DQA1*05-DQB1*02* encoding the DQ2 heterodimer [3, 4]. Usually, the alpha and beta chains of this heterodimer are encoded together on a *DRB1*03* (DR3) haplotype. However, they may also be encoded in *trans* with the *DQA1*05* allele usually on *DRB1*11, DRB1*12,* or *DRB1*13* haplotypes, and the *DQB1*02* allele usually on a *DRB1*07* (DR7) haplotype [5, 6]. The DQ8 heterodimer (coded by *DQA1*03-DQB1*0302*), carried on a *DRB1*04* (DR4) haplotype, is commonly encoded for by celiacs who do not carry the DQ2 heterodimer [7]. Only a small number of celiacs

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carry neither the DQ2 nor DQ8 heterodimers, many of whom have been reported to encode just one chain of the DQ2 heterodimer [8].

For those celiacs without the DQ2 heterodimer, a role for the *DRB4* gene, found on almost all *DRB1*04* and *DRB1*07* haplotypes and encoding the DR53 molecule, has been suggested [9, 10]. However, the hypothesis has not been supported in more recent studies [11–13]. The *DPB1*0101* allele has been proposed to affect the level of susceptibility attributed to the DR3 haplotype, although this may be due to linkage disequilibrium with other loci [14]. The role of *DPB1* has not been studied in DQ2 and DQ8 heterodimer negative patients. In type 1 diabetes, also a disease associated with the DR3-DQ2 and the DR4-DQ8 haplotypes, the degree of susceptibility conferred by the DR4-DQ8 haplotype is determined by the subtypes of *DRB1*04,* with an additional influence from HLA-B [15, 16]. Indeed, HLA class I alleles have also been suggested to have an additional influence on the risk attributable to DR3-DQ2 haplotypes in celiacs, with *A*01-B*08*-DR3-DQ2 haplotypes more often transmitted to celiacs than other DR3-DQ2 haplotypes from Finland [17, 18].

We have postulated that by examining a large series of celiacs without the known HLA-DQ risk genotypes, we may reveal associations with class I or additional class II HLA loci. Investigations of the HLA associations of celiacs without the DQ2 heterodimer have been limited by the rarity of such patients. The multinational collaboration that forms this European genetics consortium has provided a unique opportunity. Here, we present the largest such study to date, with extensive genotyping of both HLA class II and class I genes.

METHODS AND MATERIALS

Subjects

Celiac patients (1008 patients) were recruited from Finland, France, Italy, Norway, Sweden, and the UK. All patients included were unrelated, some were from multiplex families (one patient taken from a family containing two or more celiacs) and some from simplex families (where only one member is known to be affected). The groups contributed patients as follows: Finnish group, 100 celiac patients; French group, 92 celiac patients; Italian group, 302 celiac patients; combined Norwegian and Swedish group, 326 celiac patients; UK group, 188 celiac patients. The selection of celiac patients in all groups reflected the need of each center to recruit families for other studies of genetic linkage and association. Emphasis was therefore sometimes placed on recruiting families with multiple coeliac members, and families for whom both parents were available for donation of DNA. For the present study, only one member from each family was included from multiply affected families, although this was not always the proband. It must be noted, therefore, that although all 1008 coeliacs included in this study are unrelated, these do not represent a truly randomly selected sample of the European population; this is a genetic and not epidemiological study. All patients fulfilled the revised ESPGAN criteria [19] for the diagnosis of celiac disease from the UK, Italian, Norwegian, Swedish, and French groups. Finnish patients had a firm diagnosis of celiac disease by local gastroenterologists [20]; however, clinical details were not available from all patients to confirm adherence to the ESPGAN criteria. Approval from the regional ethics committees for each participating center was obtained, as was written informed consent from every participating subject. Genomic DNA was extracted from whole blood by all partners.

HLA Typing Strategy: Stage 1

All patients were genotyped for the presence or absence of the DQ2 heterodimer, defined as the presence of both *DQA1*05* and *DQB1*02* alleles. In the French group some *HLA-DQA1* alleles were inferred from the known linkage disequilibrium in that population after typing for *DRB1* and *DQB1* alleles.

HLA Typing Strategy: Stage 2

Having identified the patients not carrying the DQ2 heterodimer in *cis* or *trans,* this group was analyzed in more detail. All patients were typed for *DRB1, DQA1,* and *DQB1* alleles except some French patients in whom *DQA1* alleles were inferred from known linkage disequilibrium in that population with *DRB1* and *DQB1* alleles. Most patients without either the DQ2 or DQ8 heterodimers were also typed for *DRB3-5, DPB1, A, B,* and *C* alleles, and some for *DRB1*04* subtypes.

HLA Typing Methods

Finland. Initial HLA typing was performed using the Dynal AllSet+ SSP DQ low- resolution kit (Dynal Biotech, Oslo, Norway). *DRB1* types were determined by using two microsatellite markers (*DQCAR* and *DQCA-RII*), which are known to be in strong linkage disequilibrium with *DRB1* alleles in the Finnish population [21, 22]. All uncertain cases were typed by LiPA-DRB kits (Murex and Innogenetics, Ghen, Belgium). Typing for *HLA-A, -B, -DRB1-5, -DQA1, -DQB1,* and *-DPB* was performed using commercial kits (Dynal Biotech; Innogenetics; Olerup SSP, Qiagen, Leusden, The Netherlands; Pel-Freez Clinical Systems, Brown Deer, WI, USA.

France. All HLA typings were performed by the reverse hybridization principle using the Murex and Innogentics

TABLE 1 Number of patients included from each center with basic grouping by HLA type

	Index cases	DQ2 negative (all)	DQ2 negative DQ8 positive	DQ2 negative DQ8 negative
France	92	12	6	6
Italy	302	49	17	32
Finland	100	9	5	4
Norway and Sweden	326	28	17	11
UK	188	23	15	8
Total	1008	121	60	61

DQ2 is defined as the presence of the heterodimer *DQA1*05 DQB1*02;* DQ8 is the heterodimer *DQA1*03 DQB1*0302.*

INNO LiPA *HLA-DQB1* and *DRB1* kits. *DRB3-5, DPB1,* and HLA class I typing was not performed.

Italy. Initial HLA typing was performed with the Dynal AllSet+ SSP DR and DQ low-resolution kits, and some also with Dynal AllSet+ SSP *DQA1* typing kit (Dynal Biotech). Higher resolution typings were with the LiPA *HLA-A,* LiPA *HLA-B,* LiPA *HLA-DRB,* LiPA *HLA-DQB,* LiPA *HLA-DPB* (Innogenetics), and the Dynal AllSet+ SSP *DRB1-5, DQA1, DQB1*03,* and *DQB1*06* kits (Dynal Biotech).

Norway-Sweden. HLA-DQA1 and *DQB1* genotyping was done by polymerase chain reaction–sequence-specific oligonucleotide (PCR-SSOP), as previously described [23]; *HLA-A* and *-B* were typed by Dynal Reli SSO (Dynal Biotech) and DR Low, *DRB1,* and *DPB1* subtyping kits were used (Olerup SSP).

UK. All HLA class I and II typing performed by PCR-SSOP as previously described [24, 25] except *HLA-DPB1* typing was performed by the reverse hybridization technique using the Murex INNO LiPA *HLA-DPB1* kit (Innogenetics).

Statistics

Significance was determined by applying a simple binomial test. A p value < 0.05 was considered significant.

RESULTS

The numbers of patients without the DQ2 heterodimer are shown in Table 1. The proportion of patients negative for the DQ2 in these nonrandom population samples was higher in the Southern European population (France and Italy) than the Northern European population (Finland, Norway, Sweden, UK) (15.5% vs 9.8%; $p = 0.007$). This difference reflected the increased proportion of southern European patients without either the DQ2 or DQ8 heterodimers compared with northern Europeans (9.6% vs 3.7%; $p = 0.0001$), rather than an increase in the fraction of DQ2 negative, DQ8 positive patients (5.8% vs 6%; $p = 0.6$).

Twenty-nine of 60 patients negative for DQ2 but positive for DQ8 heterodimers, were DQ8 (*DQA1*03-DQB1*0302*) homozygous. Eleven of 60 DQ2 negative, DQ8 positive patients carried a *DRB1*07-DQB1*02* haplotype. The HLA class II haplotypes of the patients without either the DQ2 or DQ8 heterodimers are illustrated in Table 2. The great majority encode for one-half of the DQ2 heterodimer, *i.e.,* either *DQA1*05* or *DQB1*02.* This was the clearest association represented by all but four patients. DQ5 (*DQA1*01-DQB1*05*) was carried by 23/61, although most also carried one-half of the heterodimer. Of those 4 patients lacking either DQA1*05 or DQB1*02, three carried *DQA1*01-DQB1*05.* No associations were seen with other *DQ* heterodimers. No associations were seen with *DRB3* or *DRB5* (data not presented). Table 3 demonstrates the HLA class I types in patients without the DQ8 or DQ2 heterodimers (only 41/61 underwent class I typing). In this table the *DRB1* types are shown for reference. *HLA-B*44* was present in 19/41 cases. However, *B*44* tended to occur on *DRB1*07* haplotypes (14/19 cases) and, thus, any association with DQ2 and DQ8 heterodimer negative celiac disease may only reflect linkage disequilibrium (which is exceptionally strong in the HLA complex [26]) with *DRB1*07,* and *DQB1*02.* Apart from the high frequency of *HLA B*44,* there is no clear HLA class I association among this group. Table 4 illustrates the clinical details of the 4/1008 patients who encoded neither the DQ8 heterodimer nor either chain of the DQ2 heterodimer. These 4 patients clearly have unusual HLA-DQ genotypes for celiacs, however this table shows that these do not have strikingly similar or atypical presentations of celiac disease. All fulfilled the ESPGAN diagnostic criteria for celiac disease [19].

The *DRB4* genotypes are depicted in Table 2 alongside the corresponding DR and DQ types for the patients who are negative for both DQ2 and DQ8 heterodimers (in some cases where full typing has not been done, the presence or absence of *DRB4* is inferred from the *DRB1* type – this is indicated in the table). None of these typed patients encoded for the *DRB4* gene except for those with a *DRB1*07* haplotype. The distribution of *DRB1*04* alleles is illustrated in Table 5 – the alleles are quite evenly distributed among DR4-DQ8 haplotypes. *HLA-DPB1* typing was performed in 42 of the DQ2 and DQ8 negative patients; no clear association was seen with any particular *DPB1* type (data not shown). It is notable that the *DPB1*0101* allele was only found in 4/42 tested at the *DPB1* locus. Similarly, among the DQ2 and DQ8 heterodimer negative celiacs *DPB1*0301*

TABLE 2 HLA class II haplotypes of patients without either DQ2 or DQ8 heterodimers

Individual	DRB1*	DQA1*	DQB1*	DRB1*	DQA1*	DQB1*	DRB4*
Italy 1	0701	0201	02	0701	0201	02	0101 (+/−0103)
Italy 2	0701	0201	02	0701	0201	02	0103
Nor/Swe 1	0701	0201	02	0701	0201	02	0101, 0103
Nor/Swe 2	0701	0201	02	0701	0201	02	0101, 0101
UK 1	0701	0201	02	0701	0201	02	0101/3
France 1	0701	0201	02	0701	0201	02	Positive*
Italy 3	0701	0201	02	0701	0201	02	0101 (+/−0103)
Italy 4	0701	0201	02	0701	0201	02	Positive*
Finland 1	0701	0201	02	0701	0201	02	0103
Italy 5	0701	0201	02	0701	0201	02	0101 (+/−0103)
Italy 27	0701	02	02	0701	02	02	Positive*
Italy 6	0701	0201	02	0801	0401	04	0103
Italy 7	0701	x	02	14	x	05	0103
Italy 8	0701	0201	02	10	0105	05	0101
Italy 9	0701	0201	02	0701	0201	0302/3	0101 (+/−0103)
Nor/Swe 3	0701	0201	02	0701	0201	0303	0101, 0103
Nor/Swe 7	0701	0201	02	0101	0101	0501	0101
Nor/Swe 6	0701	0201	02	1401	0101	0503	0103
Nor/Swe 4	0701	0201	02	1501	0102	0602	0101
UK 2	0701	0201	02	0401	0301	0301	0101/3
UK 3	0701	0201	02	0103	0101	0501	0101-4
UK 4	0701	0201	02	1301/2	0103	0603	0101-4
Italy 10	0701	0201	02	0801	0401	04	0103
France 2	0701	0201	02	0701	0201	03032/33/06	Positive*
Italy 11	0701	0201	02	0101	0101	0501	0103
France 3	0701	0201	02	16	0102	0502	Positive*
Italy 12	0701	0201	02	1601	0102	0502	0101
Italy 13	0701	0201	02	1401	0104	0503	0101
France 4	0701	0201	02	01	0101	05011	Positive*
Finland 2	0701	0201	02	0801	0401	0401/2	0101
Finland 3	0701	0201	02	09	0302	0303	0103
Finland 4	0701	0201	02	1501	0102	0602	0103
Italy 14	0701	0201	02	1503	0102	0602	0103
Italy 15	0701	0201	02	04	0303	03011	0101 (+/−0103)
Italy 16	0701	0201	02	0901	0302	03032	0103
Italy 28	0701	02	02	1001	01	0501	Positive*
Italy 29	0701	02	02	01	01	0501	Positive*
Italy 30	0701	02	02	1301	01	0603	Positive*
Italy 31	0701	02	02	02	01	0502	Positive*
Italy 32	0701	02	0202	08	04	0402	Positive*
Italy 17	0701	0201	02	1404	0104	05031	0103
Italy 18	1104	0505	0301	1104	0505	0301	Negative*
Italy 19	11	0505	0301	11	0505	0301	Negative*
Italy 20	1201	0505	0301	13	0505	0301	Negative*
Italy 21	05	0505	0301	0701	0201	0303	0101
Italy 22	05	0505	0301	1601	0102	05	Negative*
Italy 23	1104	0505	0301	1601	0102	0502	Negative*
UK 5	11	0501	0301	0701	0201	03032	0101/3
France 6	11	0501	0301	15	0102	0602	Negative*
France 7	11	0501	0301	13	0102	0609	Negative*
UK 6	12	0501	0301	15	0101-4	0602/11	Negative
UK 7	1303/4	0501	0301	0101/2/4	0101	0501	Negative
Nor/Swe 11	1101	05	0301	0701	0201	0303	0103
Nor/Swe 12	1104	05	0301	1501	0102	0602	Negative
Nor/Swe 8	1303	05	0301	0101	0101	0501	Negative
Nor/Swe 9	1201	05	0301	1001	0101	0501	Negative
Nor/Swe 10	1101	05	0301	1001	0101	0501	Negative
Italy 24	14	01	05	01	0104	05	Negative*
Italy 25	0804	0401	0402	0102	0101	0501	Negative*
UK 8	08	0401	0401-2	0101/2/4	01	0501	Negative
Italy 26	0401	0303	0301	0701	0201	0303	0101 (+/−0103)

Parts of the DQ2 heterodimer and the DRB4 genotype are highlighted.

* In these patients the *DRB4* gene was not specifically typed for, rather it was inferred from *DRB1* and DQ genotypes. "Nor/Swe" indicates combined Norwegian and Swedish samples. "x" implies result unknown.

TABLE 3 *HLA-A* and *-B* types of celiac patients negative for both DQ2 and DQ8 heterodimers

Patient	HLA-A*		HLA-B*		HLA-DRB1*	
UK 3	11	26	44	35	07	01
UK 2	01	02	44	44	07	04
UK 1	02	03	44	44	07	07
Nor/Swe 2	29	74	44	44/47	07	07
Nor/Swe 11	03	03	44	57	07	11
Nor/Swe 4	02	02	44	40	07	15
Italy 13	01	03	44	38	07	14
Nor/Swe 3	02	29	44	27	07	07
Italy 3	01	26	44	13	07	07
Italy 1	x	x	44	13	07	07
Nor/Swe 1	02	23	44	13	07	07
Nor/Swe 7	24	29	44	07	07	01
Finland 2	03	29	44	15	07	08
Finland 4	02	68	44	13	07	15
UK 8	03	32	44	18	08	01
UK 6	01	02	44	40	12	15
Nor/Swe 9	03	32	44	35	12	01
Nor/Swe 8	02	03	44	35	13	01
Italy 24	02	29	44	35	14	01
Italy 12	02	02	40	51/52/78	07	16
Italy 21	01	23	37	50	07	05
Nor/Swe 12	03	31	27	51	11	15
UK 7	01	68	27	35	13	01
Italy 23	02	11	18	35	11	16
Italy 8	02	24	14	35	07	10
Italy 25	x	x	14	38	08	01
Italy 9	02	02	13	15	07	07
Finland 1	02	30	13	13	07	07
Italy 10	11	24	13	39	07	08
Finland 3	02	02	13	51	07	09
Nor/Swe 6	02	03	13	56	07	14
UK 5	02	30	13	15	07	11
Italy 26	01	02	08	41	07	04
Italy 16	x	x	07	13	07	09
UK 4	03	29	07	14	07	13
Italy 19	01	03	07	18	11	11
Italy 18	03	11	07	18	11	11
Nor/Swe 10	11	29	07	49	11	10
Italy 2	01	01	x	x	07	07
Italy 5	23	24	x	x	07	07
Italy 20	26	32	x	x	12	13

DRB1 alleles are given for reference (replicated from Table 2); "x" implies result unknown.

was present in only 2/39 patients. A further 3 patients were reported as possible carriers, this allele being one of several possible alleles detected by the typing technique used.

DISCUSSION

This European collaborative project has allowed HLA associations to be studied among the largest reported series of coeliac patients without the DQ2 or DQ8 heterodimers. The most noteworthy result was that of the 61/1008 patients identified without the DQ2 or DQ8 heterodimer, 57 of them encoded one half of the DQ2 heterodimer. This has confirmed the findings of much smaller series [8, 12, 13, 27, 28], and has in itself important clinical implications. HLA-DQ typing has been proposed as part of a celiac disease screening protocol for high risk groups [29] although only recommended as circumstantial evidence for diagnosis [30]. We recommend that clinicians should not classify HLA types only as DQ2/8 heterodimer positive or negative, but must also consider the presence of one half of the DQ2 heterodimer as compatible with a diagnosis of coeliac disease. It is very rare for celiac disease to occur without one of these genotypes, although the possibility should not be excluded.

TABLE 4 The clinical details of the four patients without the DQ8 heterodimer or at least one part of the DQ2 heterodimer

Patient	Age at onset	Age at diagnosis	Symptoms	Family history?
Italy 24	1 year	2 years	Diarrhea, failure to thrive, vomiting	None
Italy 25	Asymptomatic	6 years	Asymptomatic (family screening)	Sibling with celiac disease
UK 8	50 years	50 years	Diarrhea	Sibling with coeliac disease
Italy 26	1.5 years	2 years	Diarrhea, vomiting, failure to thrive, abdominal pain.	Sibling with coeliac disease

Five smaller series of DQ2 and DQ8 heterodimer negative coeliacs [7, 8, 12, 31, 32] have together reported 9 patients without either *DQA1*05* or *DQB1*02* alleles, 6 of whom carried a *DQB1*06* allele. None of our 4 patients without DQ2, DQ8 heterodimers, *DQA1*05* alone nor *DQB1*02* alleles, carried a *DQB1*06* allele. Indeed, as this is one of the more common *DQB1* alleles in Caucasoid populations [33, 34], the previous occurrence of this allele in this highly selected group is unlikely to represent a true disease association. Furthermore, a role for *DQA1*0101-DQB1*0501* (encoding DQ5) has been suggested for this subpopulation [35]. We observed that this haplotype was indeed fairly common (23/61) amongst those celiacs without the DQ2 or DQ8 heterodimers, and was carried by 3/4 of those in the group without DQ2 or DQ8 heterodimers or one half of the DQ2 heterodimer. However, as *DQA1*01-DQB1*05* is a frequent haplotype in Caucasoid populations this may represent a selection-induced exaggeration of the allele frequency.

Some studies have found an association of the *DPB1*0101* allele with celiac disease possibly as a marker of an extended higher risk DR3-DQ2 haplotype [14, 36]. The finding that *DPB1*0101* is unusual in DQ2 and DQ8 heterodimer negative celiacs is consistent with no independent role for the *DPB1*0101* allele in celiac disease susceptibility. Similarly, the *DPB1*0301* allele was unusual amongst DQ2 and DQ8 heterodimer negative celiacs in this study, this allele has been previously found with increased frequency amongst celiacs in southern Italy [37], most of whom were DQ2 heterodimer positive.

A role for the *DRB4* gene that encodes the DR53 molecule has been proposed as a susceptibility factor in DQ2 heterodimer negative celiac disease. This suggestion was based on the observed association of the *DRB4* gene with DQ2 heterodimer negative celiac patients, and on the finding that the DR53 molecule is capable of selectively binding α-gliadin derived peptides [9]. The *DRB4* gene is usually only present on *DRB1*04, DRB1*07,* and *DRB1*09* haplotypes in European Caucasian populations. Of the 16 patients without either DQ2 or DQ8 heterodimers, or a *DRB1*07* haplotype, none had a *DRB1*09* haplotype – that is, none of these patients encoded for the *DRB4* gene (in 7 of these, this was confirmed by *DRB4* typing, in the others it was inferred from known linkage disequilibrium between DR and DQ alleles). This, in agreement with other recent studies [11, 12], suggests that the association of DQ2 heterodimer negative celiac disease with the *DRB4* gene is probably secondary to linkage disequilibrium with the HLA-DQ genes on the *DRB1*04* and

TABLE 5 The distribution of *DRB1*04* alleles amongst patients negative for the DQ2 heterodimer but positive for the DQ8 heterodimer

Center	Number of patients	Number of *DRB1*04* haplotypes	*DRB1*04* alleles				
			0401	*0402*	*0403*	*0404*	*0405*
Finland	4	6	2	0	3	1	0
Italy	10	13	2	3	2	2	4
UK	12	17	4	3	0	7	3
Total	26	36	8	6	5	10	7

French and Norwegian-Swedish patients were not typed.

*DRB1*07* haplotypes, rather than a primary association. The *DRB1*04* allele subtypes of the DR4-DQ8 positive patients are shown for reference in Table 5. These are fairly evenly distributed, although whether any alleles reveal a statistically significant association to celiac disease cannot be analyzed without a suitable control group, which is not available at the present time.

It is interesting that of the 60 DQ2 negative, DR4-DQ8 positive patients the haplotype combinations were 29 DR4/DR4; 11 DR4/DR7 and 20 DR4/DRx (where x is not DR3, DR4, or DR7). This gives the impression of an excess incidence of DQ8 homozygosity in this group and a possible small increase in the frequency of DR7. This parallels findings in DQ2 positive patients, as homozygosity for *DQB1*02* is associated with increased risk of celiac disease [5, 23, 31], which is found for both DR3/DR3 and DR3/DR7 haplotype combinations. Accurate statistical analysis of the data presented would be very complicated, but this is an observation that should be tested further in studies designed to address this issue using suitable control populations.

By looking only at patients negative for the DQ2 and DQ8 heterodimers, we postulated that extensive HLA typing might reveal other class I or class II associations with celiac disease. In fact, no *HLA-DRB1, -DPB1,* or *-A* associations were seen. As discussed, the *DRB4* association and a weak association with *HLA-B*44* in this group are probably both secondary to linkage disequilibrium with the *DRB1*07-DQB1*02* haplotype. Of course, in patients that do encode for DQ2 or DQ8 heterodimers, there may be additional factors within the HLA region that further modify disease risk, however this study was not designed to address this issue. Also, we have not investigated the role of the non-classical-HLA genes within this region in modifying disease susceptibility in DQ2 negative patients, such as the *MICA*5.1* allele that was recently reported to be increased in this group [13].

In conclusion, this large series of celiac patients without the DQ2 heterodimer has not revealed evidence for classical HLA class I or II gene associations outside HLA-DQ. Rather, we have demonstrated that the overwhelming majority of celiac patients carry the DQ2 or DQ8 heterodimers, or encode one chain of the DQ2 heterodimer (*i.e., DQA1*05* or *DQB1*02* but not both), underlining the primary importance of *HLA-DQ* molecules in the genetic susceptibility to celiac disease.

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APPENDIX

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