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SHORT COMMUNICATION

UNUSUAL HLA TYPING IN CELIAC DISEASE

R. GRILLO*, F. PETRONZELLI*, P. FERRANTE*, B. MORA*, M. BONAMICO†, M.C. MAZZILLI*
*Department of Experimental Medicine, University of Rome "La Sapienza", Italy
†Department of Pediatrics, University of Rome "La Sapienza", Italy

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Accumulating evidence shows that susceptibility to celiac disease (CD) is conferred by the HLA-DQ2 heterodimer encoded by the DQA1*0501 and DQB1*0201 alleles usually inherited in *cis* with the DRB1*03 haplotype or in *trans* with the DRB1*05 and DRB1*07 haplotypes (Sollid *et al.*, 1989; Hall *et al.*, 1992; Lundin *et al.*, 1993; Sollid and Thorsby, 1993). Although only a few cases of DQ2 negative CD patients have been described, most of them seemed to be DR4 positive encoding the DQ8 molecule (De Marchi *et al.*, 1984; Tosi *et al.*, 1986: Spurkland *et al.*, 1992; Tighe *et al.*, 1993). Furthermore, Congia and colleagues (1994) recently reported three DQ2 and DQ8 negative cases among Sardinian patients encoding the DQ(α1*0101,β1*0501) heterodimer, suggesting a possible role for the DQ5 molecule in CD susceptibility.

We have previously reported (Mazzilli *et al.*, 1992) that 92% of CD Italian children carried the DQ2 heterodimer in combination with DR3 or DR5/7 alleles.

In this report we describe HLA typing of 13 CD patients who carry neither DR3 nor DR5/7, characterised for the -DRB1,-DQA1,-DQB1 genes by PCR-SSP (Table 1). They were selected from a total of 143 patients collected over a period of 4 years by the Pediatrics Clinic of the "La Sapienza" University Hospital in Rome. Diagnosis of CD was made according to the new European Society for Pediatric Gastroenterology and Nutrition (ESPGAN) criteria (Walker-Smith *et al.*, 1990). Eleven out of the 13 patients were unrelated children while the remaining two were an affected sib pair (n. 2 and n. 6). Typing of these patients' families has been undertaken so that the haplotypes described are derived by segregation. The 13 patients are divided into three groups. The first consists of 4 patients encoding the DQ2 molecule on rare haplotypic combinations. One patient (n. 1) carried the DQA1*0501 and the DQB1*0201 alleles in *cis* on DR13 haplotype. The remaining three patients encoded the dimer at risk in *trans*, one with a DR5,DQA1*0501; DR9,DQB1*0201 (n. 2) and the other two (n. 3 and n. 4) with DR6,DQA1*0501;DR7,DQB1*0201 combinations. The finding of patients with rare haplotypes carrying the DQ2 heterodimer, further supports the hypothesis of a primary association of CD with this particular DQ heterodimer.

The second group includes 6 DR4 positive patients. The genomic typing of the samples revealed that all but one carried a DQ8 heterodimer (α1*0301,β1*0302), while

Correspondence to: Prof. M. Cristina Mazzilli, Dipartimento di Medicina Sperimentale, Viale Regina Elena, 324, 00161 Roma, Italy. Fax: 06-4454820.

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Table 1. HLA-haplotypes, clinical forms and age at the diagnosis of DR 3, DR5/7 negative patients.

	Haplotypes						Disease	
ID number	DR	DQA1*	DQB1*	DR	DQA1*	DQB1*	Type	Age
1	13	0501	0201	14	0101	0501	A	8.0
2*	9	0302	0201	11	0501	0301	T	4.3
3	6	0501	0301	7	0201	0201	A	11.1
4	6	0501	0301	7	0201	0201	T	1.0
5	4	0301	0302	7	0201	0201	S	21.0
6*	4	0301	0302	9	0302	0201	T	1.8
7	4	0301	0302	7	0201	0303	T	3.0
8	4	0301	0302	11	0501	0301	T	8.7
9	4	0301	0302	11	0501	0301	T	12.0
10	4	0302	0301	7	0201	0201	T	2.8
11	1	0101	0501	11	0501	0301	T	1.0
12	1	0101	0501	7	0201	0201	T	4.6
13	10	0104	0501	7	0201	0201	T	1.4

*patients n. 2 and n. 6 are sib pair.
S=Silent; T=Typical; A=Atypical.
The age at the diagnosis is reported in years.

the remaining one was DQ7 positive (α1*0302,β1*0301) (Figure 1). To our knowledge, this is the first case of a CD patient carrying a DR4,DQ7 haplotype. Overall, the presence of DQA1*0302 in this patient confirms the diversity of this haplotype from that usually found in CD. The finding that most DR4 positive patients carry the DQ8 molecule indicates that this heterodimer confers susceptibility to CD, as reported by other authors (Lundin *et al.*, 1994a; 1994b).

The third group is represented by three DQ2 and DQ8 negative patients. Two of them carry DR1 and the other one DR10 as first haplotype and DR5 or DR7 as second haplotype. Interestingly, a report from Congia and colleagues (1994) described identical HLA-typings in three patients belonging to the Sardinian population. These data from two different populations seem to support the possible association with CD of DQ5 heterodimer (α1*0101,β1*0501), encoded by both DR1 and DR10 haplotypes. A recent report (Fodgell and Olerup, 1994) described a new mutation that splits DQA1*0101 alleles into DQA1*0101 and DQA1*0104 in disequilibrium with DR1 and DR10, respectively. These two alleles can be distinguished only by a G-A transition in exon I. We characterised this mutation among the patients described above, confirming the associations reported in the healthy population. Assuming a role for DQ5 dimer as the presenting molecule of gliadin, it is likely that the mutation in exon I does not affect the binding of this dimer to the peptides.

The disease type and age at diagnosis of the unrelated children and the sib-pair are also described in Table 1.

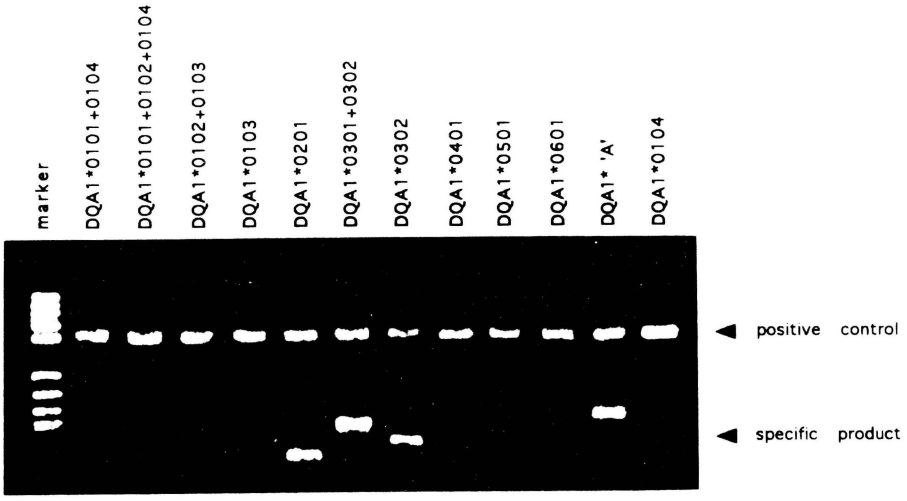

Figure 1. Genomic typing by Dynal DQA1 SSP kit of patient n. 10. The inferred typing was DQA1*0201,*0302. The DQA1*0302 is amplified by specific primers recognizing the mutation at codon 7 that distinguishes this allele from DQA1*0301. A further mutation at codon 160 (C → A transition) distinguishes DQA1*0301 from the DQA1*0302 allele. Using probes (Fernandez-Vina *et al.*, 1994) able to recognize the two sequences, the typing was confirmed.

Twelve out of the 13 CD patients showed a symptomatic form of CD, 10 with typical and 2 with atypical presentation, while the remaining one was a female diagnosed at 21 years with a silent form of the disease. The data presented here show no significant correlation between clinical forms of the disease and HLA typing.

In conclusion, HLA-DQA1 and -DQB1 possibly represent the most important susceptibility genes even in DR3 and DR5/7 negative patients. Furthermore, the DQ5 dimer previously described in three Sardinian patients seems to play a role in the pathogenesis of CD also in subjects not native to Sardinia. The data reported in this study and in other reports let us suggest the existence of a gradient of risk conferred by three different DQ dimers: DQ2>DQ8>DQ5.

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