Brief Definitive Report

EVIDENCE FOR A PRIMARY ASSOCIATION OF CELIAC DISEASE TO A PARTICULAR HLA-DQ α/β HETERODIMER

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Celiac disease (CD) is a malabsorptive disorder precipitated in genetically susceptible individuals by ingestion of gluten. A significant part of the disease predisposition maps to the HLA class II region, but the primary HLA association is not known (1). DR3 seems to be a susceptibility allele together with any other DR allele while DR7 is associated to CD almost only in combination with DR3 or DR5 (2, 3).

The HLA-DR3DQw2 haplotype demonstrates the strongest association to CD (1–5). We investigated whether DR3DQw2 negative patients might share parts of the genetic information of this HLA haplotype but encoded in *trans* position. When we compared amino acid sequences of different DQα and DQβ chains we found that the DQα chain encoded by the DR3DQw2 haplotype is identical to that encoded by DR5DQw7 (6, 7), while the DQβ chain of the DR3DQw2 haplotype is identical to that of DR7DQw2 except for a single amino acid difference in the second domain (8, 9; Lee, J. S., personal communication). The pairing of α and β chains seems to be determined by amino acid residues located in the first domains (10). Thus, individuals who are DR5DQw7/DR7DQw2 heterozygous may by transcomplementation express the same (or almost the same) DQ α/β heterodimers as those formed by DQA1 and DQB1 genes in *cis* position on the DR3DQw2 haplotype (see Fig. 1). As a first step to confirm this hypothesis we typed 94 CD children with synthetic DQA1 and DQB1 allele-specific oligonucleotide (ASO) probes. Here we report that all except one of these CD patients carry DQA1 and DQB1 genes that may encode the same DQ α/β heterodimer.

Materials and Methods

Patients and Normal Controls. The CD patients were 94 unrelated Norwegian children. The diagnosis of CD was made according to the ESPGAN criteria (11). 56 subjects attending to the local blood bank served as normal controls.

Serological HLA Typing. HLA-DR typing was performed on class II–positive cells enriched from peripheral blood by immunomagnetic isolation (12). Highly selected International Workshop (IWS) antisera were used for typing.

Allele-specific Oligonucleotide (ASO) Probes. The DQA1-2 probe with the sequence 5'-TTA-ATCAGACTGTTCAAGTT is complementary to the sense strand (amino acid positions 72–78 according to numbering used in reference 13) of the DQA1 gene of DR3DQw2 and DR5DQw7

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(6, 7). This probe was kindly synthesized by E. Hornes (AL Lab, Oslo, Norway). The DQB1-2 probe is identical to the sense strand (amino acid positions 26–33) of the DQB1 gene of DR3DQw2 and DR7DQw2 (8, 9) and has the sequence 5'-TGTGAGCAGAAGCATCTATA. This probe was obtained from Genetic Designs (Houston, TX). The probes were enlabeled to a specific activity of 1.5×10^7 cpm/pmol by T4 polynucleotide kinase using γ-[^{32}P]ATP.

Oligonucleotide Hybridization. DNA was prepared from citrated blood or from EBV-transformed B cells (one patient and all IWS cell lines). Digestion of DNA (7 μg) was done with Taq I restriction endonuclease (4 U/μg DNA) as recommended by the manufacturer (Amersham Corp., Amersham, UK). Restriction fragments were separated by gel electrophoresis using 0.8% agarose. The gels were then dried and hybridized using conditions mainly as described previously (14). Hybridizations were performed at the following temperatures: DQA1-2, 50°C and DQB1-2, 54°C. The allele-specific reactivity of the probes was tested on Taq I digests from a selected panel of 31 10th IWS cell lines. The DQA1-2 probe hybridized to a single 4.6-kb DNA fragment only from DR3DQw2, DRw11DQw7, DRw12DQw7, DRw14DQw7, and DRw16DQw7 homozygous cells, whereas the DQB1-2 probe hybridized to a single 1.5-kb fragment only from DR3DQw2 and DR7DQw2 cells.

Results and Discussion

Serological DR typing revealed that 90 of 94 (95.7%) of the CD patients were DR3$^+$ of which 27 were heterozygous DR3/7. Three of the four DR3$^-$ patients were DR5/7 (two DRw11/7, one DRw12/7), while the remaining patient was DR4/w6.

We then tested Taq I digests of DNA from 94 CD patients and healthy controls with the ASOs. The DQA1-2 and DQB1-2 probes hybridized to DNA from all CD patients except the DR4/DRw6 patient (Table I, Fig. 2). In comparison, these probes hybridized to DNA from 14 out of 56 (25.0%) healthy controls (Table I). The data suggest that almost all CD patients share a particular combination of a DQA1 and a DQB1 gene; i.e., they may share a particular *cis*- or *trans*-encoded DQ α/β heterodimer (Fig. 1). A similar conclusion was recently reached by others based on studies of restriction fragment length polymorphism (15). The results of serological DQ typing of CD patients are also compatible with our findings (3); DR5/7 CD patients always carry a DR7DQw2 haplotype, while DR3/7 CD patients may either carry a DR7DQw2 or a DR7DQw3 haplotype as the disease susceptibility DQ genes are represented by the DR3DQw2 haplotype.

In our material 95.7% of the CD patients were DR3$^+$ and 3.2% were DR5/7 heterozygous. The low frequency of DR5/7 correlates with a low frequency of this phenotype in the normal Norwegian population (0.9%, our unpublished observation). In contrast, an Italian study has revealed that almost 30% of the CD patients are DR5/7$^+$, whereas the corresponding frequency in the normal population ap-

TABLE I

Frequencies of Reactivity of the DQA1-2 and DQB1-2 ASO Probes with DNA from CD Patients and Normal Controls

Reactivity with probes	CD patients (n = 94)		Normal controls (n = 56)	
	+	%	+	%
DQA1-2	93	98.9	17	30.4
DQB1-2	93	98.9	21	37.5
DQA1-2 and DQB1-2	93	98.9	14	25.0

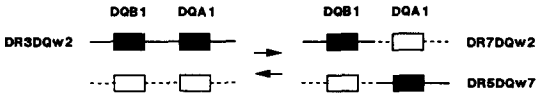

FIGURE 1. Schematic representation of structural and possible evolutionary relationship between HLA-DQA1 and -DQB1 genes of the DR3DQw2, DR5DQw7, and DR7DQw2 haplotypes. Boxes with equivalent shading represent identical or almost identical genes.

pears to be ~8% (3). Parallel results have been obtained in a Spanish study (2). The frequency of DR3 and DR5/7 among CD patients thus seems to be correlated to the normal frequency of these phenotypes. This may at least in part explain the different DR associations observed in various populations.

Some few DR7$^+$ CD patients have been reported to be negative for DR3 or DR5 (2, 3). It needs to be investigated whether these individuals carry the DQA1 and DQB1 genes conferring high susceptibility for CD. There has also been reported CD patients who are DR3$^-$ and DR7$^-$. These patients have all been DR4$^+$ (16). This was also the case of our DR3$^-$ and DR7$^-$ patient, whose DNA did not hybridize with the DQA1-2 and DQB1-2 probes. The reason why these "odd" patients are susceptible to CD is at present unknown. One possibility might be that two different gluten peptides are able to precipitate CD. One peptide may interact with the particular DQ α/β heterodimer suggested by our studies, while another may interact with a DR4-associated class II molecule. Evidence for a similar mechanism has recently been given for the MHC-associated disorder experimental allergic encephalomyelitis (EAE) in mice (17).

Several reports have recently indicated an association between CD and certain genomic DP polymorphisms (18, 19). At present it is not clear whether both DQ and DP genes might contribute to susceptibility to CD or whether the DP associations are secondary to the DQ association.

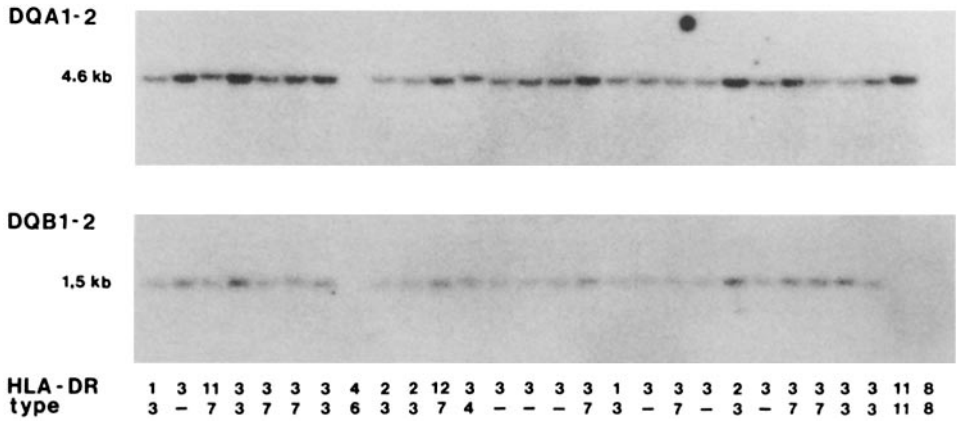

FIGURE 2. Hybridization of the DQA1-2 and DQB1-2 ASO probes to Taq I restriction fragments of genomic DNA from 25 CD patients and three homozygous cell lines. The homozygous cell lines were tested in the outer three right lanes: DR3/3, VAVY; DRw11/w11, JVM; and DRw8/w8, BM9. Homozygosity of DR alleles for some patients was established by family typing. "w" designations have been omitted for the DRw11, DRw12, DRw6, and DRw8 specificities.

DNA sequencing of exon regions have revealed identical nucleotide sequences of the DQA1 genes of the DR3DQw2 and DR5DQw7 haplotypes, except one substitution in the 5'-untranslated region and identical nucleotide sequences of the first domain of the DQB1 genes of the DR3DQw2 and DR7DQw2 haplotypes (6–9). This indicates a common origin of these genes. Sharing of intron polymorphisms revealed by RFLP analysis supports this assumption (20). Several reports suggest that established haplotypes have arisen by crossing over in the region between the DQA1 and DQB1 genes (7, 21). We believe that the DR3DQw2 haplotype either has contributed to the formation of, or has been formed by, the DR7DQw2 and DR5DQw7 haplotypes by a crossing over event (Fig. 1). The genetic information of the DR3DQw2 haplotype is thus reestablished in DR5DQw7/DR7DQw2 heterozygotes though the genes are split between two chromosomes. Susceptibility to CD probably depends on interaction between genes that on the DR3DQw2 haplotype are separated by the putative crossover site. Theoretically this gene interaction may involve any HLA-linked genes, for instance, regulatory genes. However, the DQA1 and DQB1 genes are very good candidates since their products form heterodimers and since they are situated close to the crossover site.

The DQα chain encoded by the DRw8DQw4 haplotype is similar but not identical to the DQα chain encoded by the CD-associated DR3DQw2 and DR5DQw7 haplotypes. DNA sequencing of the first domains have only revealed disparities in residues 69 and 75 (6, 7, 13). No excess of DR7/DRw8 heterozygotes among CD patients has been reported. The model for the three-dimensional structure of class II molecules suggests that both residue 69 and 75 are involved in binding of foreign antigen (22). Interestingly, serine at residue 75 is unique for the DQα chain of the DR3DQw2 and DR5DQw7 haplotypes. Binding of gluten peptides to class II molecules is presumably related to the pathogenesis of CD. Our data indicate that a particular DQ α/β heterodimer plays a decisive role in this respect where residues on both the α and β chains participate and where residues 69 and 75 on the α chain may have critical roles.

Summary

Typing of DNA from 94 unrelated children with celiac disease (CD) with HLA-DQA1 and -DQB1 allele-specific oligonucleotide probes revealed that all but one (i.e., 98.9%) may share a particular combination of a DQA1 and a DQB1 gene. These genes are arranged in *cis* position on the DR3DQw2 haplotype and in *trans* position in DR5DQw7/DR7DQw2 heterozygous individuals. Thus, most CD patients may share the same *cis*- or *trans*-encoded HLA-DQ α/β heterodimer.

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