On genes and inflammatory bowel disease

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Publication date
1999

Chapter 5

HLA-DR phenotypes and inflammatory bowel disease: a meta-analysis

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ABSTRACT

Susceptibility to inflammatory bowel disease (IBD) is partially genetically determined and the HLA class II genes are candidates for a role in genetic susceptibility to IBD, because their products play a central role in the immune response. Many studies have reported associations between HLA-DR phenotypes and either ulcerative colitis or Crohn's disease, but many data remain controversial. We performed a meta-analysis to estimate overall associations between HLA-DR alleles and IBD, and to establish the relative risk conferred by HLA-DR phenotypes. Medline was searched for publications reporting on the relation between IBD and HLA-DR phenotypes. Raw data were extracted by recalculating the number of phenotypes or the number of alleles of the HLA-DR main antigens. Odds ratios and confidence intervals were calculated according to the Mantel-Haenszel method. Ulcerative colitis was positively associated with DR2(15) and DR9, and negatively with DR4. For CD a positive association was found with DR7 and a negative association with DR2 and DR3. We conclude that both ulcerative colitis and Crohn's disease are associated with specific HLA-DR phenotypes. Further analysis of these phenotypes and subgroup analysis may elucidate how these alleles contribute to the susceptibility to inflammatory bowel disease.

INTRODUCTION

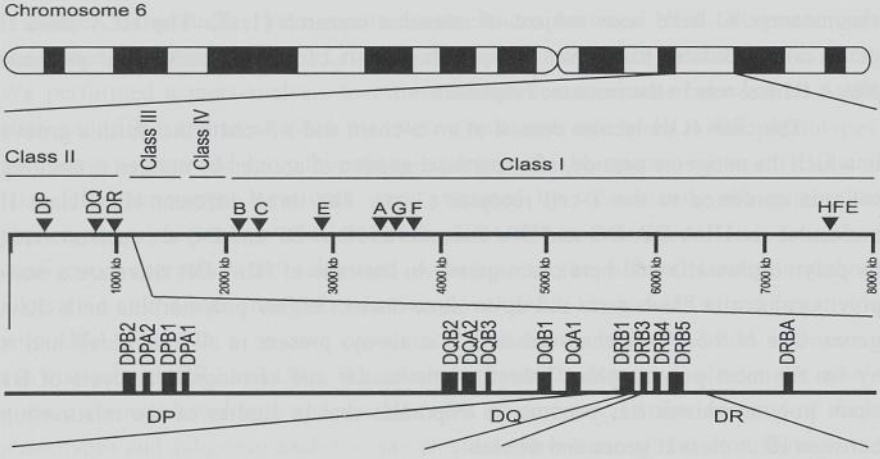
Susceptibility to inflammatory bowel disease (IBD) is partially genetically determined. Putative associations of IBD with the polymorphic genes, that are located in the major histocompatibility complex (MHC) on the short arm of chromosome 6, have been subject of intensive research (1, 2). The HLA class II genes are candidates for a role in the pathogenesis of IBD, because their products play a central role in the immune response.

The class II molecules consist of an α-chain and a β-chain that form a groove in which the antigenic peptide, after partial digestion of antigen by antigen presenting cells, is conferred to the T-cell receptor (3, 4). The three different HLA class II molecules are HLA-DP, DQ and DR. Subunits of HLA-DP and DQ are each encoded by polymorphic alfa and beta chain genes. In the case of HLA-DR there are a non-polymorphic alfa chain gene and up to three distinct highly polymorphic beta chain genes. One of these beta chain genes, B1, is always present in all individuals and is by far the most polymorphic. Therefore, molecular and serological analysis of B1 chain polymorphisms has become an important tool in studies of the relationship between HLA class II genes and disease.

Generally, patients and controls are typed for the serological main antigens, HLA-DR1 through 10, although the main antigens can be further sub-specified. The alleles are grouped by the serological phenotypic characteristics they share. In time, serological typing became more specific and subclasses were identified. Split antigens for DR2, DR3, DR5 and DR6 are DR15 and 16, DR 17 and 18, DR11 and 12 and DR13 and 14 respectively. Current nomenclature is based on molecular typing and summarizes the name of the molecule, the chain, the gene number by which it is encoded, an asterisk as an indication of molecular typing, and the number of the allele. Thus HLA-DRB1*0401 denotes an allele on the first gene defining a beta chain for the HLA-DR molecule (fig.1).

Besides the fact that the alleles can be used to study the relationship between HLA class II genes and disease, polymorphic sequences may have functional implications. Different alleles have different peptide binding characteristics, and polymorphisms that are located outside the binding site of the molecules may affect interaction with T-cells or expression of the HLA molecule (5). However, association between a HLA allele and disease does not prove such a functional relationship. The MHC region contains numerous immune related genes, and it has now become clear that the different alleles of the MHC genes are strongly linked. For example, HLA-

Figure 1 HLA-DR gene nomenclature. The HLA-DR genes are located in the major histocompatibility region on chromosome 6. The DRA gene, encoding the alfa chain of the molecule is not polymorphic. The DRB 1, 3, 4 and 5 genes are polymorphic. (A) The serological specificities of the DRB1 polymorphisms and the corresponding genotypes. (B-D) The serological specificities and the corresponding genotypes for DRB3, DRB4 and DRB5 respectively.



Serological specificity		Allele B1*
DR1		0101/0102/0104
DR103		0103
DR2	DR15	1501-1504
	DR16	1601-1606
DR3	DR17	0301
	DR18	0302-302
DR4		0401-0419
DR5	DR11	1101-1113
	DR12	1201-1203
DR6	DR13	1301-1313
	DR14	1401-1417
DR7		0701
DR8		0801-0811
DR9		0901
DR10		1001

Serological specificity	Allele B3*
DR52a	0101
DR52b	0201-0202
DR52c	0301

Serological specificity	Allele B4*
DR53	0101-0103

Serological specificity	Allele B5*
DR51	0101-0102
	0201-0203

DR3 is in linkage disequilibrium with HLA-A1, B8 and the infrequent allele of a polymorphism in the TNF promoter region (TNF-308).

Association studies have suggested a role for HLA-DR alleles in disease susceptibility or resistance to inflammatory bowel disease. Thus, HLA-DR1 (6, 7) DR4 (8-10) DR5 (8) and DR7 (7, 11) were found to be positively associated with CD. For HLA-DR2 (9, 12), DR3 (7, 11, 13) and DR8 (14), negative associations were reported. For UC, positive associations were found with DR2 (15) (6, 15-20), DR6 (21), DR12 (22) and DR103 (22) and negative associations with DR3 (20) DR4 (6, 16, 17, 21), DR6 (6) and DR7 (23). While some associations have not been confirmed, other associations were more consistently found.

Association studies are prone to false positive results, in particular when small groups are tested and when inadequate racial matching exists between controls and patients. Therefore, we have performed a meta-analysis of the literature. The aim of our study was to calculate overall associations between HLA-DR alleles and IBD.

MATERIAL AND METHODS

Publications reporting the HLA-DR main antigen frequency in healthy controls compared to either UC, CD or both were identified by searching Medline for the years 1966 through June 1998. The keywords we used were "inflammatory bowel disease", "Crohn's disease", "ulcerative colitis" and "regional" "enteritis" separately in combination with "HLA". A book chapter on genetics and IBD [2] and the reference lists of the papers found were also used as a source. Studies that exclusively addressed the relation of HLA-DR antigens in disease subgroups defined by clinical criteria or other disease markers were not included. Studies on HLA-DR sub-specificities were only included when the frequency of the main antigen could be extracted and reports on haplotype frequencies were excluded from the analysis. Full information on the phenotype frequency of at least one of the main antigens was sufficient for inclusion in the meta-analysis. When necessary, authors were contacted for additional information.

In order to compare studies that used serological typing and genotyping, we used the following rules:

1. Data on the frequency of DR17 were included in the analysis of DR3 although this ignores the existence of the rare DR18 alleles.
2. Some studies reported DR2 frequencies, whereas others report DR15 and DR16 frequencies. In case of the latter situation, we summed the given value of both

antigens, ignoring the possibility of DR15/DR16 heterozygotes. The data on DR6 and the DR13/14 split antigens were analyzed similarly.

3. Both studies on phenotype frequencies and allele frequencies were included.

The analysis required knowledge of either the number of individuals or the number of alleles. Therefore, when only percentages were given, we recalculated the original number of patients, rounding the numbers according to conventional rules. For two studies on CD odds ratios were calculated from allele frequencies instead of phenotype frequencies (7, 11). All other studies reported phenotype frequencies or both. In these cases odds ratios were calculated from the phenotype frequencies.

One study mentioned allele frequencies while the data indicated that phenotype frequencies were given because the percentages of the healthy controls were similar to the phenotype frequencies found in other studies (22). Furthermore, summing the percentages yielded a total which exceeded 100%, implicating that phenotype frequencies were given. Therefore, we handled these data as phenotype frequencies. Another study analyzed the data in relation to two different control groups, one group of healthy individuals from the same island as the patients (Kyushu Island) and a group from the general Japanese population, and we decided to include the first control group (9). Finally, one study (24) presented data that had been included in another study, which combined data from different groups (G. Semana, personal communication) (7). Therefore, the Heresbach study was excluded from the analysis.

Statistics

Odds ratios (OR) and 95% confident intervals (CI) were determined by DerSimonian and Laird method using Review Manager version 3.1 software (Update Software LTD, Oxford, England). Analysis was done using a random effect model. The etiological fraction and preventive fraction, which estimate the relative contribution of a marker to the disease susceptibility or prevention, was calculated as described (25, 26).

RESULTS

The literature search yielded 27 studies that reported on studies containing information on HLA-DR phenotype or allele frequencies in IBD patients as compared with healthy controls. The number of patients and their ethnic background are listed in table 1.

Fifteen studies on CD and HLA-DR antigens were included in the analysis. A negative association was found for DR2 (OR=0.83 CI=0.70-0.98) and DR3 (OR=0.71 CI=0.56-0.90) yielding preventive fractions of 0.04 and 0.04 respectively.

Table 1 Studies included in the meta-analysis, number of subjects and ethnic background

Study			Patients		Controls	Population
Nr	First author	Year	UC	CD		
1	*Peña*[39]	1980	-	65	148	Dutch
2	*Asakura*[18]	1982	40	-	51	Japanese
3	*Smolen*[28]	1982	30	27	25	Middle European
4	*McConnel*[23]	1983	31	-	149	British
5	*Caruso*[12]	1983	-	28	132	Sicilian
6	*Fujita*[8]	1984	-	27	231	Japanese
7	*Cottone*[31]	1985	46	-	169	British
8	*Caruso*[19]	1985	41	-	151	Sicilian
9	*Kobayashi*[16]	1990	-	30	54	Japanese
10	*Kobayashi*[10]	1990	26	-	54	Japanese
11	*Purrmann*[14]	1990	296		300	German, Caucasian
12	*Matake*[9]	1992	-	149	136	Japanese
13	*Toyoda*[6]	1993	26	74	77	American
14	*Sugimura*[17]	1993	37	-	99	Japanese
15	*Wassmuth*[40]	1993	-	109	85	Swedish
16	*Mehal*[29]	1994	42	-	64	British, Caucasian
17	*Futami*[27]	1995	59	-	150	Japanese
18	*Leidenius*[21]	1995	77	-	106	Finnish
19	*Duerr*[30]	1995	97	-	149	North American, Jewish/non-Jewish
20	*Nakajima*[41]	1995	-	90	336	Japanese
21	*Danze*[7]	1996	-	344	488	French, Caucasian
22	*Satsangi*[22]	1996	175	173	472	British, Caucasian
23	*Forcione*[13]	1996	40	42	93	North American Caucasian
24	*Reinshagen*[11]	1996	-	162	4251	German, Caucasians
25	*De la Concha*[20]	1997	107	-	200	Spanish, Caucasian
26	*Bouma*[15]	1997	59	89	2400	Dutch, Caucasian
27	*Stokkers*		70	69	420	Dutch, Caucasian

Table 2: Combined analysis of HLA-DR antigens in relation to IBD

HLA-DR antigen		Number of studies		OR (CI95%)	
Main	Split	CD	UC	CD	UC
DR1		14	13	0.90 (0.64-1.26)	1.08 (0.81-1.44)
DR2		15	17	0.83 (0.70-0.98)	2.00 (01.52-2.63)
	DR15	4	6	1.13 (0.91-1.40)	1.65 (1.22-2.25)
	DR16	4	6	0.67(0.30-1.51)	0.59 (0.30-1.23)
DR3		12	13	0.71 (0.56-0.90)	0.83 (0.63-1.09)
DR4		15	15	1.18 (0.89-1.56)	0.54 (0.43-0.68)
DR5		14	14	1.18 (0.99-1.42)	1.22 (0.93-1.60)
	DR11	6	4	1.03 (0.82-1.29)	1.09 (0.66-1.80)
	DR12	6	4	1.15 (0.75-1.77)	1.06 (0.30-4.12)
DR6		14	12	1.07 (0.89-1.27)	0.82 (0.60-1.12)
	DR13	6	5	1.23 (0.99-1.51)	0.82 (0.52-1.29)
	DR14	6	5	0.79(0.48-1.30)	0.95 (056-1.63)
DR7		12	11	1.42 (1.16-1.74)	0.87 (0.66-1.14)
DR8		14	11	1.11 (0.82-1.49)	1.03 (0.72-1.47)
DR9		12	12	1.12 (0.63-1.98)	1.54 (1.06-2.24)
DR10		9	8	1.65 (0.91-2.98)	0.55 (0.23-1.30)

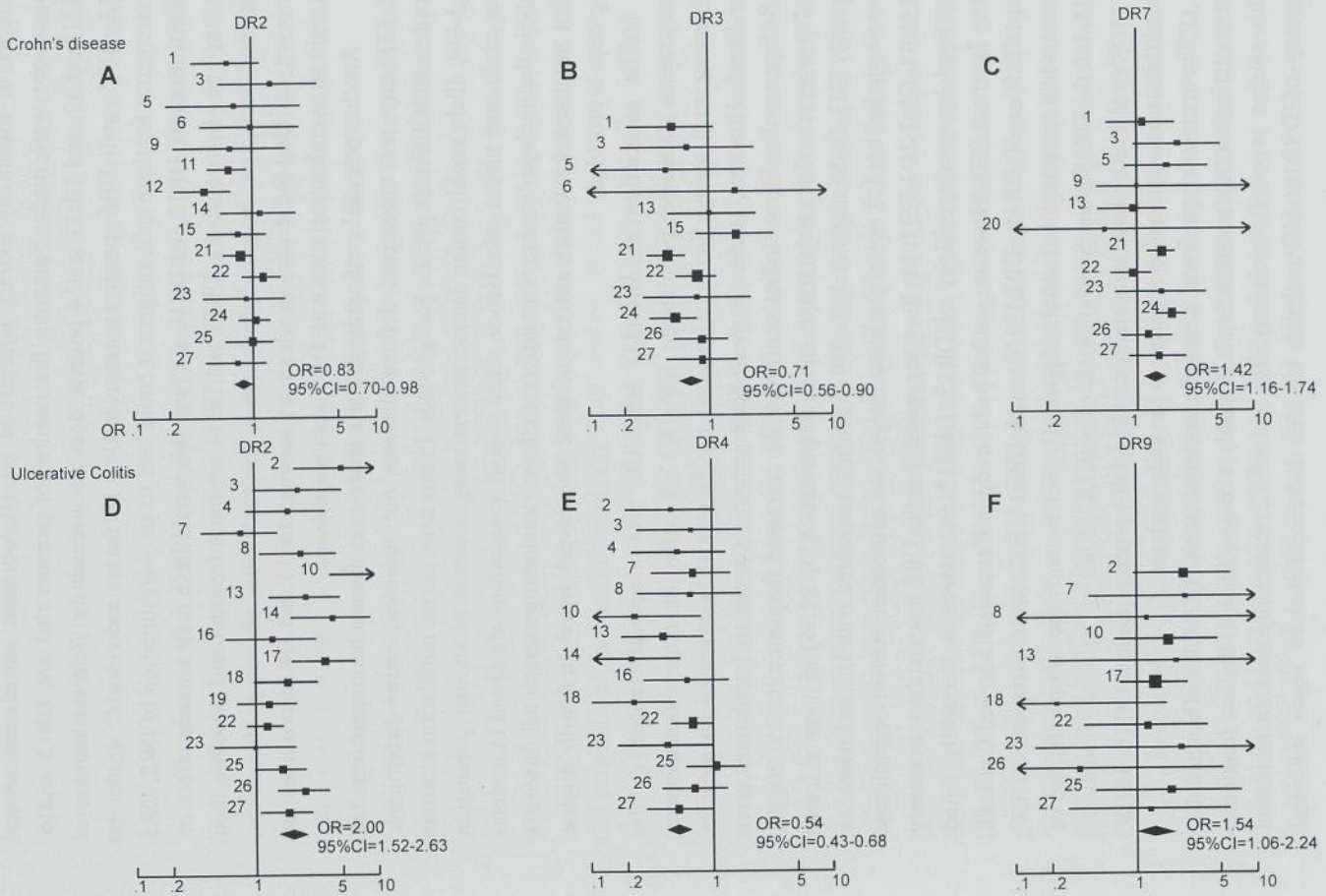
HLA-DR7 appeared to be associated with disease, with an overall OR of 1.42 (CI=1.16-1.74), resulting in an etiological fraction of 0.06 (table 2 and fig 2A-1C).

Eighteen studies qualified for the analysis of UC in relation with one or more main antigens. The repeatedly observed association with HLA-DR2 was confirmed in the cumulative odds ratio: OR=2.00 (CI=1.5-2.63, etiological fraction=0.20. (table 2 and figure 2D)) The split antigen DR15 yielded somewhat lower values (OR=1.65 CI=1.22-2.25), whereas no association was found with DR16 (table 2). An overall lower frequency was found for the DR4 antigen. (OR=0.54 CI=0.43-0.68, preventive fraction=0.15) (table 2 and figure 2E) Surprisingly, an association with DR9 was found for UC: OR=1.54, CI=1.06-2.24, etiological fraction 0.03.

DISCUSSION

Our meta-analysis confirmed a positive association of UC with DR2 and its split antigen DR15. Interestingly, an association between HLA-DR9 and ulcerative colitis was also found. In addition, HLA-DR4 appeared to be protective against UC. HLA-DR7 was positively associated with CD, and negative associations with DR2 and DR3 were noted.

Figure 2: Significant outcomes of the meta-analysis for CD (A-C) and UC (D-F)



In order to maximally extract information on HLA-disease association several concessions were made. Summing the number of patients that are DR15 and16, DR11 and 12, and DR13 and14 positive does not necessarily yield the number of DR2, DR5 and DR6 positives respectively. If many individuals would be heterozygous for these split antigens it could lead to an overestimation of DR2, DR5 or DR6. Because DR12 and DR16 are rare, this would have only a minor effect on the outcome of our analysis. DR13 and DR14 are more frequent and therefore their combined incidence may be greater than the real DR6 phenotype frequency. However, omitting the studies in which we summed DR13 and DR14 positives did not yield an overall association with DR6 (data not shown).

Another potential confounder of meta-analyses is disease heterogeneiety. UC and CD may not encompass defined diseases but represent rather heterogenic disorders with different genetic backgrounds. In this case, in one population a certain HLA-DR allele could confer disease susceptibility, whereas in another population other genes may play a role. Hence, when overall associations are found in a meta-analysis that includes studies with different ethnic, racial and geographical factors, a strong argument for further analysis of these phenotypes in relation with disease subgroups is provided.

The association of DR2(15) with UC was frequently noted in the homogeneous Japanese population (16-18, 27). Also in other homogeneous populations such as the Sicilians (19) and the Finnish (21) increased frequencies were found. However, studies in more heterogeneous Caucasian populations gave conflicting results. Some studies confirmed the increased frequency (6, 15, 20, 23, 28, 29) whereas others only found equal frequencies (13, 22,30) or even a lower frequency (31). In the meta-analysis, DR2 is firmly associated with UC and when the Japanese studies are left out of the meta-analysis the association remains significant. (OR=1.48, CI=1.24-1.76) It has been suggested that the DR2 association was mainly determined by a subspecificity of DR15, DRB1*1502 which is frequently found among Japanese (27). However, a subsequent study in Caucasians showed that both subspecificities of DR15, DRB1*1501 and DRB1*1502, were increased in UC patients when compared to controls. Apparently, not so much the subspecificities of DR15, but the DR15 phenotype is associated with UC (20).

Several studies have indicated that HLA-DR4 protects against UC (6, 16-18, 21) and this association is also apparent in the meta-analysis.

The meta-analysis found a novel association with HLA-DR9. The fact that this association was not noted before is probably a consequence of the low frequency of this antigen in most populations. Ten out of 12 studies reported an increased frequency of DR9 in UC patients, but this increase failed to reach statistical

significance. In the Japanese population, the frequency of HLA-DR9 is relatively high, and may thus be a more important factor for disease susceptibility compared to other populations. When the three Japanese studies were analyzed separately an odds ratio of 1.72 was obtained (CI=1.06-2.78), which corresponds with an etiological fraction of 0.15.

Studies on HLA-DR frequencies in CD have reported associations with DR1 (6, 7), DR4 (8-10) and recently DR7 (7-11). The latter studies also reported that DR3 conferred disease resistance (7, 11). The meta-analysis confirmed only the positive association with HLA-DR7 and a negative association for HLA-DR3. It should be noted that HLA-DR7 is in linkage disequilibrium with HLA-B44 and it remains unclear whether the association with DR7 is indirectly due to this allele.

The negative association of DR3 with CD is intriguing. Three studies have shown that the DR3 frequency is particularly low in patients with severe disease, (as indicated by the need for azathioprine treatment) (32), and in patients with peri-anal fistulas (33) In Crohn's disease, this association seems independent from linkage of DR3 with the infrequent allele of the –308 restriction fragment length polymorphism in the TNF-alfa promoter, because the frequency of this allele was not reduced in the CD patients with peri-anal fistulas (33).

The calculations of the etiological and preventive fractions should be interpreted with caution, since the HLA-DR phenotype frequencies vary among different populations and data on the age distribution of the groups that were studied are lacking (25, 26). However, these calculations can serve as an indicator for the relative contribution of the specific HLA-DR molecules to disease susceptibility. Thus, the contribution of HLA-DR to disease susceptibility for UC is relatively high (etiological fraction of 0.2 for DR2 and 0.03 for DR9), whereas for CD the contribution for DR molecules is rather modest (0.06). These findings are in agreement with data from recent linkage analyses: for Crohn's disease, linkage analysis did not find evidence for linkage of the HLA region to disease susceptibility (22, 34, 35). For UC, the attribution of the HLA region may determine most of the genetic etiological fraction (22), although this remains a matter of debate (35, 36).

The real disease susceptibility genes could be in linkage disequilibrium with the alleles identified by the meta-analysis. In CD, linkage analysis has shown an excess of TNF haplotype sharing by siblings in multiple affected families (37, 38).

In conclusion, this meta-analysis of the data from literature indicates that UC is associated with DR2(15) and DR9 and that DR4 confers protection. For CD, an association with DR7 and a negative association with DR2 and DR3 were found. The contribution of HLA-DR molecules to the pathogenesis of ulcerative colitis may be three fold larger when compared to Crohn's disease. Nonetheless, the etiological

fractions associated with these phenotypes cannot account for the total genetic contribution to disease susceptibility.

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