

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

P C F Stokkers, P H Reitsma, G N J Tytgat, S J H van Deventer

Abstract

Background—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. Multiple studies have reported associations between HLA-DR or -DQ phenotypes and either ulcerative colitis or Crohn's disease, but much of the data are still controversial.

Aims—To estimate overall associations between HLA class II phenotypes and IBD, and to establish the relative risk conferred by HLA-DR and -DQ phenotypes by meta-analysis.

Methods—Medline was searched for publications reporting on the relation between IBD and HLA class II phenotypes. Raw data were extracted by recalculating the number of phenotypes or the number of alleles of the main antigens. Odds ratios and confidence intervals were calculated according to the Mantel-Haenszel method.

Results—DR2, DR9, and DRB1*0103 were positively associated with ulcerative colitis, and a negative association was found for DR4 and ulcerative colitis. For Crohn's disease a positive association was found with DR7, DRB3*0301, and DQ4 and a negative association with DR2 and DR3.

Conclusions—Both ulcerative colitis and Crohn's disease are associated with specific HLA class II phenotypes. Further analysis of these phenotypes and subgroup analysis may elucidate how these alleles contribute to susceptibility to IBD.

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Keywords: ulcerative colitis; Crohn's disease; HLA-DR; HLA-DQ

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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 the subject of intensive research.^{1,2} The human leucocyte antigen (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 α and β chain genes. In the case of HLA-DR there is a non-polymorphic α chain gene and up to three distinct highly polymorphic β chain genes. One of these β 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 relation between HLA class II genes and disease.

Generally, patients and controls are typed for the main serological antigens, HLA-DR1–10, although the main antigens can be further sub-specified. The alleles are grouped by the serological phenotypic characteristics they share. Serological typing has become more specific and subclasses have been identified. Split antigens for DR2, DR3, DR5, and DR6 are DR15 and 16, DR17 and 18, DR11 and 12, and DR13 and 14 respectively. DQ1 was split into DQ5 and 6, DQ3 into DQ7–9. Molecular typing of HLA alleles distinguishes even more subclasses and is more reliable than serotyping. Therefore, molecular typing is generally preferred and current HLA nomenclature is based on this method. The names summarise 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 β chain for the HLA-DR molecule (fig 1).

Besides the fact that the alleles can be used to study the relation 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 an HLA allele and disease does not prove such a functional relation. 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-DR3 is in linkage disequilibrium with HLA-A1, B8, and the infrequent allele of a polymorphism in the tumour necrosis factor (TNF) promoter region (TNF-308).

Abbreviations used in this paper: CI, confidence interval; HLA, human leucocyte antigen; IBD, inflammatory bowel disease; MHC, major histocompatibility complex; OR, odds ratio; TNF, tumour necrosis factor.

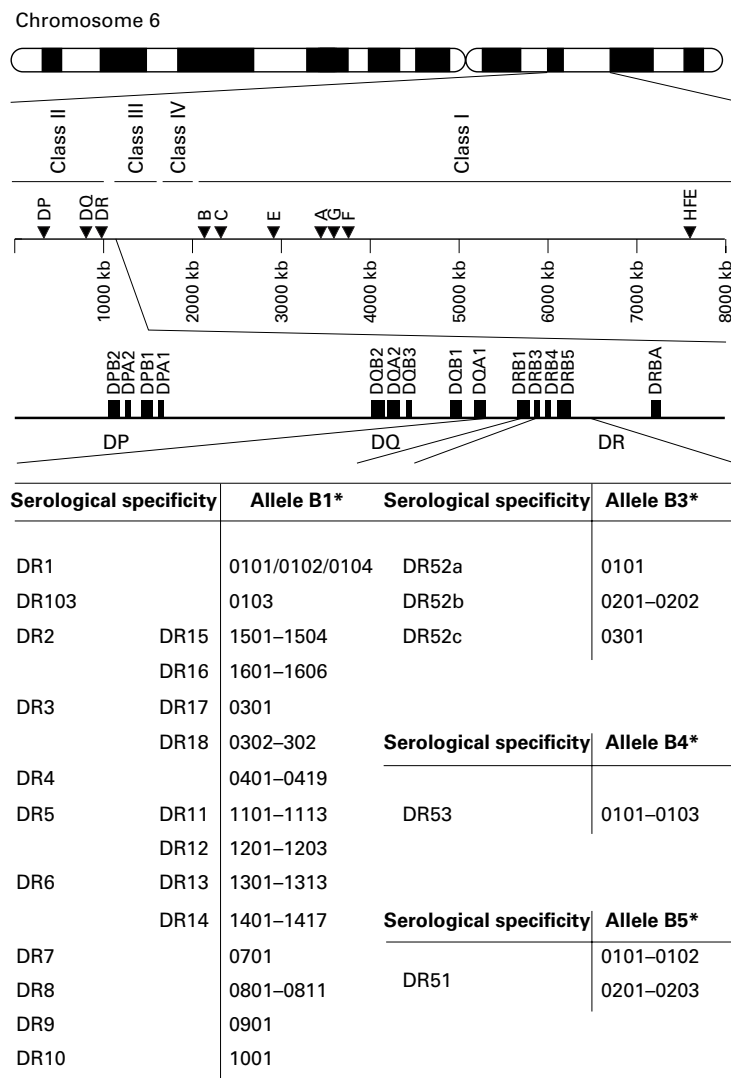


Figure 1 HLA-DR gene nomenclature. The HLA-DR genes are located in the major histocompatibility region on chromosome 6. The DRA gene, encoding the α chain of the molecule is not polymorphic. The DRB 1, 3, 4, and 5 genes are polymorphic. The serological specificities of the DRB1, DRB3, DRB4, and DRB5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

Association studies have suggested a role for HLA-DR alleles in disease susceptibility or resistance to IBD. Thus, HLA-DR1,^{6,7} DR4,⁸⁻¹⁰ DR5,⁸ and DR7^{7,11} were found to be positively associated with Crohn's disease. For HLA-DR2,^{9,12} DR3,^{7,11,13} and DR8,¹⁴ negative associations were reported. For ulcerative colitis, positive associations were found with DR2,^{6,15-20} DR6,²¹ DR12,²² and DR103²² and negative associations with DR3,²⁰ DR4,^{5,16,17,21} DR6,⁶ and DR7.²³ Although some associations have not been confirmed, others were more consistently found.

HLA-DQ antigens have less often been studied than the DR antigens: four studies have assessed DQ frequencies in patients with ulcerative colitis^{6,17,20,22} and nine in patients with Crohn's disease.^{6,7,9-11,22,25,26} ulcerative colitis has been associated with DQ2²⁰ and negatively with DQ3.¹⁷ Higher frequencies of DQ4 have been found in patients with Crohn's disease in three studies.^{9,11,25} In addition, positive associations with DQ3¹⁰ and

DQB1*0201,²⁶ and negative associations with DQ1¹⁰ and DQ6²⁵ have been reported.

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 or -DQ main antigen frequency in healthy controls compared with either ulcerative colitis, Crohn's disease, or both were identified by searching Medline for the years 1966 to June 1998. The keywords used were: inflammatory bowel disease, Crohn's disease, ulcerative colitis, and regional enteritis, separately and in combination with HLA. A book chapter on genetics and IBD² and the reference lists of the papers found were also used as a source. Studies that exclusively addressed the relation of HLA-DR/DQ antigens in disease subgroups defined by clinical criteria or other disease markers were not included. Studies on subspecificities 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:

- Data on the frequency of DR17 were included in the analysis of DR3 although this ignores the existence of the rare DR18 alleles.
- Some studies reported DR2 frequencies, whereas others reported DR15 and DR16 frequencies. In 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 analysed similarly. For DQ1 and its split antigens DQ5/DQ6 we considered this approach unsuitable, because DQ5 and DQ6 heterozygotes are frequent.
- Studies on both 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 Crohn's disease 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

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

| Study | Patients | | | Controls | Population | |
|-------|----------------------------|--------------|------|----------|------------|-----------------------------------|
| | No | First author | Year | | | |
| 1 | Peña ⁴⁸ | 1980 | — | 65 | 48 | Dutch |
| 2 | Asakura ¹⁸ | 1982 | 40 | — | 51 | Japanese |
| 3 | Smolen ³¹ | 1982 | 30 | 27 | 25 | Middle European |
| 4 | McConnell ²³ | 1983 | 31 | — | 149 | British |
| 5 | Caruso ¹² | 1983 | — | 28 | 132 | Sicilian |
| 6 | Fujita ⁸ | 1984 | — | 27 | 231 | Japanese |
| 7 | Cottone ³⁴ | 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 | Purmann ¹⁴ | 1990 | 296 | — | 300 | German, white |
| 12 | Matake ⁹ | 1992 | — | 149 | 136 | Japanese |
| 13 | Zetterquist ³⁷ | 1992 | 89 | 65 | 250 | Swedish, white |
| 14 | Toyoda ⁶ | 1993 | 26 | 74 | 77 | American |
| 15 | Sugimura ¹⁷ | 1993 | 37 | — | 99 | Japanese |
| 16 | Wassmuth ²⁴ | 1993 | — | 109 | 85 | Swedish |
| 17 | Mehal ³² | 1994 | 42 | — | 64 | British, white |
| 18 | Futami ³⁰ | 1995 | 59 | — | 150 | Japanese |
| 19 | Leidenius ²¹ | 1995 | 77 | — | 106 | Finnish |
| 20 | Duerr ³³ | 1995 | 97 | — | 149 | North American, Jewish/non-Jewish |
| 21 | Nakajima ²⁵ | 1995 | — | 90 | 336 | Japanese |
| 22 | Danze ⁷ | 1996 | — | 344 | 488 | French, white |
| 23 | Satsangi ²² | 1996 | 175 | 173 | 472 | British, white |
| 24 | Forcione ¹³ | 1996 | 40 | 42* | 93 | North American, white |
| 25 | Reinshagen ¹¹ | 1996 | — | 162 | 4251 | German, white |
| 26 | De la Concha ²⁰ | 1997 | 107 | — | 200 | Spanish, white |
| 27 | Bouma ¹⁵ | 1997 | 59 | 89 | 2400 | Dutch, white |
| 28 | Cariappa ²⁶ | 1998 | — | 58 | 93 | North American, white |
| 29 | Stokkers | | 70 | 69 | 420 | Dutch, white |

*Not used because the data on Crohn's disease (CD) are included in study 28.
UC, ulcerative colitis.

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%, implying that phenotype frequencies were given. Therefore, we handled these data as phenotype frequencies. Another study analysed 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 One study²⁶ presented data on patients with Crohn's disease from an earlier study,¹³ including additional patients with Crohn's disease and providing more detailed typing. The data from the follow up study were included in the meta-analysis. Finally, one study²⁷ presented data that had been included in another study, which combined data from different

groups (G Semana, personal communication).⁷ Therefore, the study by Heresbach and colleagues²⁷ was excluded from the analysis.

STATISTICS

Odds ratios (OR) and 95% confidence intervals (CI) were determined by the DerSimonian and Laird method using Review Manager version 3.1 software (Update Software Ltd, Oxford, UK). Analysis was done using a random effect model. The aetiological fraction and preventive fraction, which estimate the relative contribution of a marker to the disease susceptibility or prevention, were calculated as described previously.^{28, 29}

Results

The literature search yielded 29 studies that reported on studies containing information on HLA-DR/DQ phenotype or allele frequencies in IBD patients compared with healthy controls. Table 1 lists the number of patients in each study and their ethnic background.

Fifteen studies on Crohn's disease and HLA-DR antigens were included in the analysis; table 2 lists the results. A negative association was found for DR2 (OR = 0.83, CI = 0.70–0.98) (fig 2A) and DR3 (OR = 0.71, CI = 0.56–0.90) (fig 2B) yielding preventive fractions of 0.04 and 0.04 respectively. HLA-DR7 (fig 2C) seemed to be associated with disease (OR = 1.42, CI = 1.16–1.74) as was DQ4 (OR = 1.88, CI = 1.16–3.05) (fig 2D), resulting in an aetiological fraction of 0.06 and 0.04 respectively. Three studies reported on allele DRB3*0301 and meta-analysis resulted in a positive association of this allele with Crohn's disease (OR = 2.18, CI = 1.25–3.80, aetiological fraction = 0.11) (fig 2E).

Table 2 Combined analysis of HLA-DR antigens in relation to inflammatory bowel disease

| HLA-DR antigen | | Number of studies | | OR (95% CI) | |
|----------------|-------|-------------------|----|------------------|------------------|
| 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 (1.52–2.63) |
| | 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 (0.56–1.63) |
| 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) |
| DR103 | | | 3 | 3.42 (1.52–7.69) | |

CD, Crohn's disease; UC, ulcerative colitis; OR, odds ratio; CI, confidence interval.

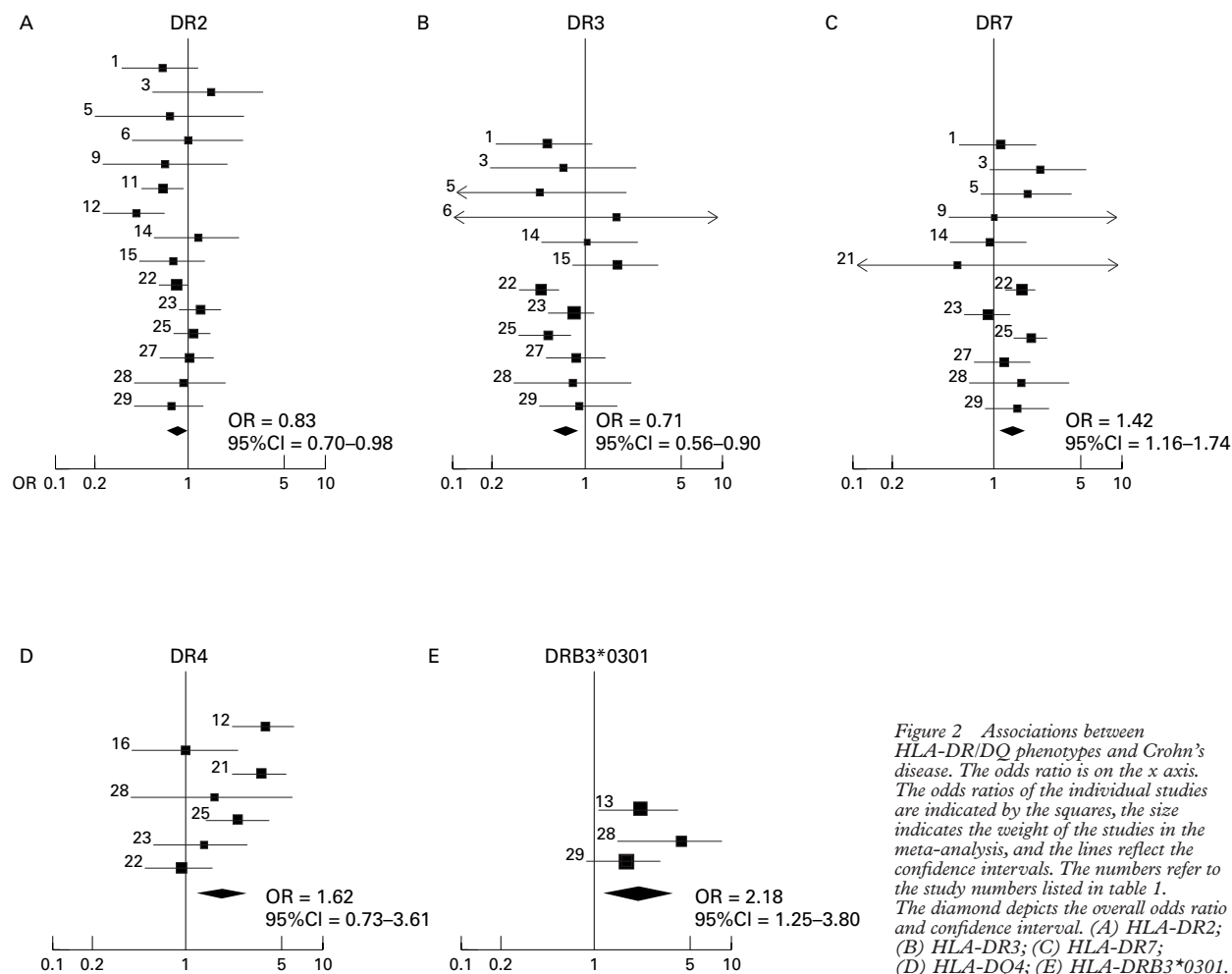


Figure 2 Associations between HLA-DR/DQ phenotypes and Crohn's disease. The odds ratio is on the x axis. The odds ratios of the individual studies are indicated by the squares, the size indicates the weight of the studies in the meta-analysis, and the lines reflect the confidence intervals. The numbers refer to the study numbers listed in table 1. The diamond depicts the overall odds ratio and confidence interval. (A) HLA-DR2; (B) HLA-DR3; (C) HLA-DR7; (D) HLA-DQ4; (E) HLA-DRB3*0301.

Table 3 Combined analysis of HLA-DQ antigens in relation to inflammatory bowel disease

| HLA-DQ antigen | | Number of studies | | OR (95% CI) | |
|----------------|-------|-------------------|----|------------------|------------------|
| Main | Split | CD | UC | CD | UC |
| DQ1 | | 3 | 1 | 0.63 (0.36–1.08) | ND |
| | DQ5 | 6 | 2 | 1.02 (0.79–1.36) | 1.30 (0.94–1.80) |
| | DQ6 | 6 | 2 | 0.83 (0.61–1.12) | 1.16 (0.61–1.12) |
| DQ2 | | 4 | 3 | 1.14 (0.73–1.78) | 0.45 (0.45–1.10) |
| | DQ3 | 2 | 1 | 1.65 (0.29–9.57) | ND |
| DQ3 | DQ7 | 3 | 2 | 0.99 (0.75–1.31) | 0.93 (0.67–1.28) |
| | DQ8 | 7 | — | 0.90 (0.71–1.14) | ND |
| | DQ9 | 6 | — | 0.98 (0.70–1.36) | ND |
| | | 7 | 1 | 1.88 (1.16–3.05) | ND |

CD, Crohn's disease; UC, ulcerative colitis; OR, odds ratio; CI, confidence interval.

Eighteen studies qualified for the analysis of ulcerative colitis in relation to one or more main antigens; table 2 lists the results. The repeatedly observed association with HLA-DR2 was confirmed in the cumulative odds ratio: OR = 2.00, CI = 1.5–2.63 and aetiological fraction = 0.20 (fig 3A). 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). Analysis of the DR15 subspecificities showed a significant association with DRB1*1502, but not with the other alleles (table 3, fig 3D–E). An overall lower frequency was found for the DR4 antigen (OR = 0.54, CI = 0.43–0.68, preventive fraction = 0.15) (fig 3B). Surprisingly, an association with DR9 was found for ulcerative

colitis: OR = 1.54, CI = 1.06–2.24 and aetiological fraction 0.03 (fig 3C). Three studies reported on allele DRB1*0103 and meta-analysis resulted in a positive association of this allele with ulcerative colitis (OR = 3.42, CI = 1.52–3.69, aetiological fraction = 0.05) (fig 3F).

Four studies on ulcerative colitis and nine studies on Crohn's disease and DQ antigens were included in the meta-analysis. Meta-analysis showed a positive association between DQ4 and Crohn's disease (OR = 1.88, CI = 1.16–3.05). No other associations were established.

Discussion

Our meta-analysis confirmed a positive association of ulcerative colitis with DR2 and allele 1502 of its split antigen DR15. Interestingly, an association between HLA-DR9 and ulcerative colitis was also found. In addition, HLA-DR4 seemed to be protective against ulcerative colitis. HLA-DR7, DRB3*0301 and DQ4 were positively associated with Crohn's disease, and negative associations with DR2 and DR3 were noted.

In order to maximally extract information on HLA-disease associations, several concessions were made. Summing the number of patients that are DR15 and 16, DR11 and 12, and DR13 and 14 positive does not necessarily

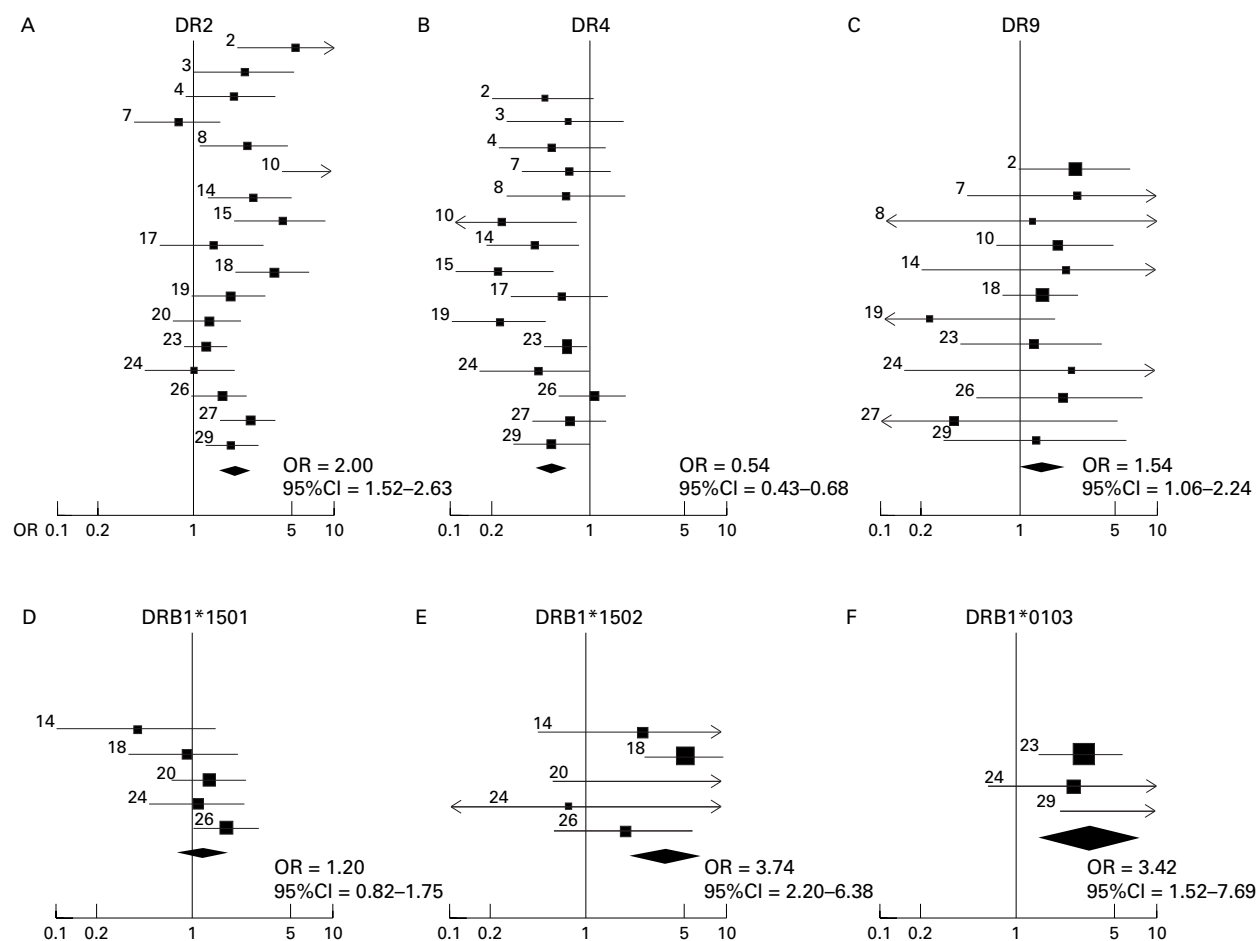


Figure 3 HLA-DR/DRB1*1501, HLA-DR/DRB1*1502, and HLA-DR/DRB1*0103 phenotypes and ulcerative colitis. The odds ratio is on the x axis. The odds ratios of the individual studies are indicated by the squares, the size indicates the weight of the studies in the meta-analysis, and the lines reflect the confidence intervals. The numbers refer to the study numbers listed in table 1. The diamond depicts the overall odds ratio and confidence interval. (A) HLA-DR2; (B) HLA-DR4; (C) HLA-DR9; (D) HLA-DRB1*1501; (E) HLA-DRB1*1502; (F) HLA-DRB1*0103.

yield the number of DR2, DR5, and DR6 positives respectively. If many individuals were 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 heterogeneity. Ulcerative colitis and Crohn's disease may not encompass defined diseases but represent rather heterogeneous 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 a transethnic analysis of these phenotypes in relation to disease subgroups is provided. Moreover, when Japanese studies and studies on white populations were analysed

separately, no gross differences in the outcome were observed.

The association of DR2¹⁵ with ulcerative colitis was frequently noted in the homogeneous Japanese population.^{16–18 30} In other homogeneous populations such as the Sicilians¹⁹ and the Finns,²¹ increased frequencies were also found. However, studies in more heterogeneous white populations gave conflicting results. Some studies confirmed the increased frequency,^{6 15 20 23 31 32} whereas others only found equal frequencies^{13 22 33} or even a lower frequency.³⁴ In the meta-analysis, DR2 is firmly associated with ulcerative colitis and when the Japanese studies are left out of the meta-analysis the association remains significant (OR = 1.51, CI = 1.20–1.90, table 4). It has been suggested that the DR2 association was mainly determined by a subspecificity of DR15, DRB1*1502 which is frequently found among the Japanese.³⁰ However, a subsequent study in whites showed that the frequencies of both subspecificities of DR15, DRB1*1501 and DRB1*1502, were increased in patients with ulcerative colitis when compared with controls.²⁰ Five studies have looked at these subspecificities and our meta-analysis shows a positive association for DRB1*1502 but not for DRB1*1501 (fig 3D–E). However, this result is biased by the high frequency of this allele in the

Table 4 Associated phenotypes in Japanese and white populations analysed separately

| HLA- | Disease | Japanese | | Whites | |
|------|---------|----------|-------------------|--------|------------------|
| | | No | OR (95% CI) | No | OR (95% CI) |
| DR2 | CD | 3 | 0.56 (0.32–0.97) | 11 | 0.89 (0.77–1.03) |
| | UC | 4 | 4.92 (3.05–7.95) | 11 | 1.51 (1.20–1.90) |
| DR3 | CD | 1 | NA | 10 | 0.70 (0.55–0.91) |
| DR7 | CD | 2 | 2.18 (0.13–37.44) | 9 | 1.40 (1.12–1.75) |
| DR9 | UC | 3 | 1.72 (1.07–2.77) | 7 | 1.50 (0.78–2.88) |
| DQ4 | CD | 2 | 3.47 (2.46–4.91) | 4 | 1.33 (0.94–1.87) |

CD, Crohn's disease; UC, ulcerative colitis; OR, odds ratio; CI, confidence interval.

Japanese study included in this analysis and the association is no longer positive when the Japanese study is omitted from the meta-analysis (data not shown). Taken together, these data suggest that in white populations, alleles other than 1502 are responsible for the association of DR2 with ulcerative colitis.

Several studies have indicated that HLA-DR4 protects against ulcerative colitis,^{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. Indeed, 10 of 12 studies reported an increased frequency of DR9 in patients with ulcerative colitis, 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 with other populations. When the three Japanese studies were analysed separately an odds ratio of 1.72 was obtained (CI = 1.06–2.78), which corresponds with an aetiological fraction of 0.15.

Finally, the HLA-DRB1*0103 allele is associated with ulcerative colitis. Three studies (one not yet published) have assessed the frequency of this rare allele in IBD patients and controls, and all reported a higher frequency in patients with ulcerative colitis.^{13 22} Others have found that this allele also predisposes to extensive disease.^{35 36}

Studies on HLA-DR frequencies in Crohn's disease 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.

DRB3*0301 has been associated with Crohn's disease²⁶ and we have identified three studies (including one not yet published) that report on the phenotypic frequency of this allele.^{26 37} Meta-analysis of these data shows a significant association between Crohn's disease and B3*0301. This allele is in tight linkage disequilibrium with DRB1*1302 in whites.³⁸ Therefore the association may either be due to one of these alleles or due to another allele in linkage disequilibrium with the DRB1*1302–B3*0301 haplotype.

The negative association of DR3 with Crohn's disease 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),³⁹ and in patients with perianal fistulas.⁴⁰ 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- α promoter, because the frequency of this allele was not reduced in the patients with Crohn's disease with perianal fistulas.⁴⁰

The association of Crohn's disease with DQ4 is no longer significant when the two Japanese studies are omitted from the analysis. The DQ4 phenotype is common among the Japanese and may therefore constitute a more important risk factor in this population.

The calculations of the aetiological and preventive fractions should be interpreted with caution, as the HLA-DR phenotype frequencies vary among different populations and data on the age distribution of the groups that were studied are lacking.^{28 29} 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 ulcerative colitis is relatively high (aetiological fraction of 0.2 for DR2, 0.05 for DR103, and 0.03 for DR9), whereas for Crohn's disease the contribution for DR molecules is smaller (0.06 for DR7 and 0.11 for DRB3*0301). These findings are in agreement with data from recent linkage analyses: for ulcerative colitis the attribution of the HLA region may determine most of the genetic aetiological fraction, whereas for Crohn's disease the role of the HLA region seemed limited.²² The aetiological fractions that we found cannot account for the total genetic risk on IBD. Genome scans indicate that non-MHC genes may play a role as well. Linkage of disease susceptibility to loci on chromosomes 1, 2, 4, 3, 7, 12, 16^{41–43} has been reported, but replication of linkage has only been obtained for the loci on chromosomes 12 and 16.^{44–47}

The frequency of HLA class II alleles varies between different populations and selection bias may underlie some of the associations found by the meta-analysis. For example, the association with DR7 is not noted when the white and Japanese populations are analysed separately (table 4). This is not surprising because DR7 is very infrequent among the Japanese population and only two studies have assessed its frequency. Therefore, the aetiological fraction of DR7 is very low for the Japanese population (due to the low prevalence of DR7). Conversely, the DR9 association with ulcerative colitis and the DQ4 association with Crohn's disease are not detected when only white populations are included in the analysis. Most likely, this is also a consequence of the low frequency of these phenotypes in the white population.

In conclusion, this meta-analysis indicates that ulcerative colitis is associated with DR2,¹⁵ DR9, and DRB1*0103 and that DR4 confers

protection. For Crohn's disease, an association with DR7, DRB3*0301, and DQ4 and a negative association with DR2 and DR3 were found. The contribution of HLA-DR molecules to the pathogenesis of ulcerative colitis may be threefold larger when compared with Crohn's disease. None of the aetiological fractions associated with these phenotypes can account for the total genetic contribution to disease susceptibility.

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