Genetic markers in clinically well defined patients with ulcerative colitis (UC)

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SUMMARY
Results of genetic association studies in UC are conflicting. We propose that the power of candidate gene studies will increase when disease heterogeneity is taken into account. Phenotype frequencies of molecularly defined HLA-DR alleles, polymorphisms in the tumour necrosis factor-alpha (TNF-α), lymphotoxin-alpha (LT-α), IL-1 receptor antagonist (IL-1Ra) and IL-1β genes were determined in 98 clinically well characterized UC patients with a mean period of follow up of 10 years, and ethnically matched healthy controls (HC). The alleles HLA-DRB1*0103 (phenotype frequency 6% versus 0·2%; P = 0·0002; odds ratio (OR) 27·6) and DRB1*15 (41% versus 26%; P = 0·001; OR = 2·0, compared with HC) were associated with overall disease susceptibility. Subgroup analysis revealed that DRB1*15 was only increased in females (53% versus 24%; P < 0·0001; OR = 3·5), but not in males. With regard to disease localization, all DRB1*0103+ patients had extensive disease (P < 0·002; OR = 33·5), and DRB1*15 was found in 59% of females with extensive colitis (P < 0·0001; OR = 4·4). DRB1*0103 was significantly increased in patients undergoing colectomy (P < 0·0002; OR = 84). No association between overall disease susceptibility and the cytokine gene polymorphisms were found. Subgroup analysis revealed several significant associations, but most did not retain significance when corrected for multiple comparisons. However, a noticeable finding was that haplotype TNF-C was significantly associated with progression in extent of disease (P = 0·003; OR = 20·4). This study provides additional evidence for the role of DRB1 alleles in the susceptibility to UC, and supports the hypothesis that these alleles may determine the severity of the disease. The cytokine gene polymorphisms evaluated in this study do not seem to be strong risk factors for the overall disease susceptibility in UC, but may be involved in determining the severity of the disease.

Keywords HLA ulcerative colitis heterogeneity cytokines polymorphism

INTRODUCTION
There is substantial evidence that genetic factors play a role in the predisposition to inflammatory bowel disease (IBD), i.e. UC and Crohn’s disease (CD) [1—4]. However, the relevant genes have not been identified. Genes of the MHC are excellent candidate genes because of their role in the immune response, and the strong associations that exist in other immune-mediated diseases. Studies on HLA associations in IBD, however, have yielded inconclusive results. An increased prevalence of HLA-DR2 (DRB1*15) in UC has been observed in several studies, especially from Japan [5—9]. No conclusive data on this association in European Caucasian patients exist, however. We recently observed an increased prevalence of DRB1*15 in a small group of Dutch UC patients [10], and an association with this allele was also found in a study from Spain [11]. A study from the UK, however, showed that the alleles DRB1*0103 and DRB1*12, but not the DRB1*15 allele, were increased in their patient population [12]. In favour of a role for the HLA alleles in disease susceptibility, Satsangi et al. reported linkage of UC to the HLA-DRB1 locus using non-parametric linkage analysis [12].

Cytokines play a key role in the initiation, regulation and effectuation of the immune response. The genes that encode...
these proteins might therefore be another group of candidate genes for UC. Mansfield et al. found that allele 2 of the IL-1 receptor antagonist (IL-1Ra) is increased in patients with (extensive) UC [13]. Although we found no significant association between disease in general and this allele, we could confirm that allele 2 was significantly increased in those patients with extensive colitis [14]. Interestingly, it was subsequently found that in IBD, carriers of the IL-1Ra allele 2 are frequently non-carriers of allele 2 of the IL-1β TaqI polymorphism, suggesting that an imbalance in the IL-1Ra/IL-1β ratio may contribute to disease susceptibility in IBD [15]. Tumour necrosis factor-alpha (TNF-α) and TNF-β or lymphphotoxin-alpha (LT-α) are cytokines that play a central role in the immune reaction. The location of their genes within the MHC class III region has prompted much speculation on the role of these cytokines in HLA-associated diseases. The central role of TNF-α in IBD has recently been shown by the clinical benefit of anti-TNF treatment in CD patients [16]. We previously studied four polymorphisms in the TNF region. A small, but significant decrease of the uncommon allele 2 at position −308 in the promoter region of the TNF-α gene was found in UC [17]. Louis et al. found this allele to be decreased, albeit non-significantly, in patients with CD, but not in UC patients [18].

Several explanations exist for the different and inconclusive results from studies regarding associations with HLA alleles and cytokine gene polymorphisms. Small sample size, controls that were not ethnically matched, and different HLA typing techniques (serological versus molecular) may all have contributed to the inconclusive and conflicting results. However, one of the most important explanations might be that most of these studies did not take into account disease heterogeneity. From the clinical point of view, UC is a heterogeneous disease with marked differences in clinical behaviour, response to treatment and prognosis. Moreover, during the course of their disease, individual patients vary considerably in clinical behaviour. It has been observed that >50% of patients will have further progression of their disease during follow up [19]. As these different clinical subgroups of patients may have a different genetic susceptibility, or alternatively, if the clinical behaviour may be influenced by a different genetic background, it is not surprising that the various studies have yielded conflicting results, as in most studies only unselected groups of patients were investigated. We propose that the power of genetic association studies may increase when disease heterogeneity is taken into account. This assumption is supported by recent findings [12,20]. Thus, no association with the DRB1*15 allele was found in an unselected group of patients from the UK [12], but subsequently a significantly increased prevalence of this allele was found in a subgroup of patients from the same hospital undergoing colectomy [20]. The authors also observed an association with the DRB1*0103 allele. The strength of the association with DRB1*0103, as estimated by the odds ratios, was stronger in their selected study population than in their unselected population. To emphasize the importance of disease heterogeneity in genetic association studies, we can add another observation. We previously studied HLA-DR associations in an unselected group of patients with CD and found no significant association with any HLA allele tested [10]. However, when we investigated in a subsequent study these alleles in a selected population of patients with fistulizing disease, we observed that the DRB1*03 allele was almost absent [21].

The aim of the present study was two-fold. In first instance we studied the relation between HLA and cytokine gene polymorphisms and detailed clinical phenotype. Subsequently, we investigated the possible interplay between the various genetic markers previously found to be associated with UC.

**MATERIALS AND METHODS**

**UC patients**

Ninety-eight randomly selected UC patients (51 females, 47 males) were included in this study. Part of this study population had been typed previously for some of the markers under investigation [10,14,15,17]. All patients were unrelated Dutch Caucasians. The diagnosis of UC was based on conventional clinicopathological criteria as described by Lennard-Jones [22]. The localization of gut involvement was defined as proctitis, left-sided (up to the splenic flexure), or pancolitis (beyond the splenic flexure), based on endoscopy and histology. Clinical data were collected retrospectively at two time points: when patients visited the Academic Hospital Vrije Universiteit for the first time (at a mean time after diagnosis of 3.6 years, range 0–30 years), and at their latest visit (after a mean follow up of 10.2 years, range 0–32 years). Mean age at diagnosis was 33 years (range 8–79 years). At the time of diagnosis, 32 patients had distal disease, whereas 66 had extensive colitis (46 left-sided colitis and 20 pancolitis). At follow up, 17 patients had distal disease and 81 extensive disease (47 left-sided colitis and 34 pancolitis). Eighteen patients showed an increase in extent of disease during follow up (15 from proctitis to left-sided and three from proctitis to pancolitis). At follow up, 24 patients had been colectomized. Fifteen UC patients had a positive family history for IBD, among whom five had a first degree relative with IBD. With regard to extra-intestinal manifestations, two patients developed sacro-iliiiitis, two pyoderma gangrenosum, one uveitis, one aphthous stomatitis, and one patient had erythema nodosum. Two patients also suffered from primary sclerosing cholangitis.

**Control group**

A group consisting of 2400 unrelated healthy Dutch Caucasian blood donors serologically typed for HLA served as controls. Controls were recruited from the same region as the patients. Part of this control group was also typed for the subtypes of DR1 (DRB1*0101/2/4 and 0103), DR2 (DRB1*15 and DRB1*16), DR5 (DRB1*11 and DRB1*12) and DR6 (DRB1*13 and DRB1*14). In particular, HLA-DRB1*15 was determined in 1576 healthy controls (HC), and the DRB1*0103 allele was studied in 420 HC.

A group of 98 HC was typed for polymorphisms in the genes encoding TNF-α, LT-α, IL-1β and the IL-1Ra.

**DNA isolation, polymerase chain reaction amplification, and dot-blotting for HLA typing**

Genomic DNA was extracted from EDTA anti-coagulated peripheral blood using a standard proteinase K digestion and phenol/chloroform extraction procedure. Amplification of the second exon of HLA-DRB1 genes was performed by polymerase chain reaction (PCR). Dot blot analysis of amplified DNA was carried out using the procedures [23] and biotin-labelled sequence-specific oligonucleotide probes (SSO) as previously described [24].

**Cytokine gene polymorphisms**

A single-stranded conformation polymorphism method was used for the detection of bi-allelic polymorphisms at both positions −308 and −238 in the TNF-α gene, as previously described [25].

PCR amplification with primers located in the 5′ untranslated
region and third intron of the LT-α was used for typing the NcoI restriction fragment length polymorphism (RFLP) [26] as well as the AspHl RFLP [27].

We previously observed that the alleles of these four bi-allelic polymorphisms occur in a restricted number of combinations denoted haplotypes TNF-C, -E, -H, -I and -P (Fig. 1) [17,28].

PCR amplification and TaqI digestion of the polymorphic site within exon 5 of the IL-1β gene were performed and analysed as previously described [15].

The region within the second intron of the IL-1Ra gene that contains variable numbers of an identical tandem repeat (VNTR) of 86 base pairs was amplified by PCR and analysed as previously described [13–15].

Statistical analysis

Statistics were calculated using Instat version 2.02 (Graphpad Software, San Diego, CA). Phenotype frequencies were compared between the study groups by means of 2×2 table analysis using χ² (with Yates’ correction) or Fisher’s exact test. We also tested the possibility of ‘dose–response’ relationships across the categories non-carrier, heterozygous carrier, and homozygous carrier using the χ² test for trend. In order to investigate interaction, i.e. to assess whether differences between estimated odds ratios were larger than expected by chance alone, we performed an exact homogeneity test, using the StatXact software package [29]. To correct for multiple comparisons in various subgroups, the P values were corrected using the Bonferroni correction where appropriate. On subgroup analysis, we tested those markers that were increased in the UC population in general: DRB1*0103, DRB1*15, haplotype TNF-C, and the IL-1Ra allele 2. For correction of the number of comparisons, the following parameters were taken into account: disease localization, progression in extent of disease, need for operation, medication, family history and extra-intestinal manifestations. Taking into account these parameters, a P value <0·005 was considered statistically significant.

To show the strength of the associations, we have included P values, odds ratios (OR) and the 95% confidence intervals (CI) in the text.

RESULTS

Associations with overall disease

HLA-DRB1. The phenotype and allele frequencies of the HLA-DR alleles are given in Table 1. From one patient, no HLA-data were available. Two alleles were found to be significantly associated with disease in general: DRB1*0103 and DRB1*15.

The phenotype frequency of DRB1*0103, which is extremely low in the Dutch population, was significantly increased in UC patients (6% versus 0·2%; P = 0·0002; OR = 27·6; 95% CI = 3·3–232).

We previously observed in a small group of UC patients that the DRB1*15 allele is increased [10]. We therefore first confirmed that this allele was increased in the new group of patients as well. We then calculated the phenotype frequency of this allele in the total group of 98 patients, and observed that this allele was significantly increased when compared with controls (41% versus 26% in HC; P = 0·001; OR = 2·0; 95% CI = 1·3–3·1).

Focusing on DRB1*15, when comparing non-carriers, carriers and homozygous carriers, we found a significant trend across these categories (χ² for trend = 19; P < 0·0001), confirming previous observations in a small group of patients [10].

The association with DRB1*15 was found to be very significant in female patients. Twenty-seven (53%) of female UC patients were carriers of the DRB1*15 allele versus 24% of female HC (P < 0·0001; OR = 3·5; 95% CI = 2·0–6·0). In contrast, no association was found in male UC patients. Thirteen male patients (28%) were carriers of this allele versus 27% of male HC. The homozygosity test revealed that the difference between males and females is significant (P = 0·01).

TNF gene polymorphisms. We previously studied polymorphisms in the TNF genes in a small group of patients and controls [17]. In the present study, this population was extended to 98 patents and 98 controls. In accordance with our previous observations, none of the TNF haplotypes was found to be associated with disease in general, as shown in Table 2. In the present group of patients, TNF-C haplotype was somewhat increased in UC patients, but the difference did not reach significance (36% versus 26%; P = 0·2; OR = 2·0; 95% CI = 0·9–3·0). The TNF haplotypes were equally distributed among females and males.

IL-1Ra and IL-1β gene polymorphisms. In previous studies, we found no significant association between disease in general and carriage of either allele 2 of the IL-1β gene or allele 2 of the IL-1Ra gene [14,15]. In the present cohort of patients, the phenotype frequency of the IL-1Ra allele 2 was 50% in UC patients versus 42% in HC (P = 0·3; OR = 1·6; 95% CI = 0·8–2·4). The distribution among males and females was equal. The IL-1β allele 2 was not significantly increased either, although we observed the previously described imbalance in the distribution of the alleles of the IL-1Ra and IL-1β gene polymorphisms in this group of UC patients (results not shown) [15].

Interaction between the genetic markers in UC. Several genetic markers have previously been found to be associated with UC. To our knowledge, however, it has never been investigated whether these genes act in conjunction in the susceptibility to the disease. Both DRB1*15 and IL-1Ra allele 2 are found in approx. 45% of patients. We calculated the phenotype frequencies of IL-1Ra allele 2 in the DRB1*15-positive and -negative subgroups and vice versa. The frequency of carriers of IL-1Ra allele 2 did not differ between DRB1*15-positive and -negative patients (60% versus 44%). Similarly, the frequency of DRB1*15 in carriers and non-carriers of the IL-1Ra allele 2 was not different (49% versus 33·3%).

With regard to the TNF haplotypes and the IL-1β allele 2, only the TNF-P haplotype, which is in linkage disequilibrium with the DRB1*15 allele, was decreased in the DRB1*15 subgroup.

HLA-DRB1 and cytokine gene polymorphisms in relation to clinical characteristics

Extent of disease. Seventeen patients (12 females, five males)
had distal disease, whereas 81 (39 females, 42 males) had extensive disease at a mean follow-up time of 10.2 years. The DRB1*0103 allele was significantly increased in patients with extensive colitis when compared with controls. In fact, all carriers of this allele had extensive disease at follow up ($P < 0.0001$; OR = 33.5; 95% CI = 4–283 compared with HC). In contrast, none of the patients with proctitis was carrier of this allele ($P = 1.0$; OR = 0.1; 95% CI = 0.005–3.2).

The phenotype frequency of the DRB1*15 allele was 29% in patients with proctitis, and did not differ significantly from HC. Of those patients with extensive colitis, 44% were DRB1*15+ ($P = 0.007$; OR = 2.2; 95% CI = 1.4–3.5). This association was highly significant in females (59%; $P < 0.0001$; OR = 4.4; 95% CI = 2.3–8.6), but not in males (Fig. 2).

Two patients (12%) with distal colitis were carriers of the TNF-C haplotype ($P = 0.0002$ compared with HC). In extensive colitis, 41% of patients were carriers of this haplotype ($P = 0.007$; OR = 2.2; 95% CI = 1.1–3.8), but this association did not retain significance after correction for the number of comparisons.

The phenotype frequency of the IL-1Ra allele 2 was somewhat higher in patients with extensive colitis (53%) compared with patients with distal colitis (35%) and controls, although the difference did not reach statistical significance. However, when we investigated the relation between carriership of allele 2 and the trend over the localizations proctitis, left-sided colitis and pancolitis, the association was significant ($\chi^2$ for trend = 6.5; $P = 0.01$).

Progression in extent of disease. At follow up, 18 patients had an increase in their extent of disease (15 from proctitis to left-sided, and three from proctitis to pancolitis). Fourteen patients had only proctitis and the disease did not progress during the same follow-up period of observation.

The DRB1*15 allele was found in five patients with stable proctitis and eight with progressive disease ($P = 0.001$). It must be noted, however, that the follow-up time in those five patients was significantly shorter than in the eight patients with progressive disease (3–6 versus 10.5 years; $P < 0.05$).

With regard to the TNF haplotypes, 11 patients with progressive disease were carriers of the TNF-C haplotype, whereas only

### Table 1. HLA-DR phenotype and allele frequencies in 97 patients with UC and 2400 controls

<table>
<thead>
<tr>
<th>HLA-DRBI*</th>
<th>HC (%)</th>
<th>UC (%)</th>
<th>UC versus control OR (95% CI)</th>
<th>HC (%)</th>
<th>UC (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>01 (0101/2/4)</td>
<td>20</td>
<td>21</td>
<td>22</td>
<td>1.0 (0.6–1.7)</td>
<td>10</td>
</tr>
<tr>
<td>0103</td>
<td>0.2</td>
<td>6</td>
<td>6</td>
<td>27.6 (3.3–232)†</td>
<td>0.1</td>
</tr>
<tr>
<td>15</td>
<td>26</td>
<td>40</td>
<td>41</td>
<td>2.0 (1.3–3.1)††</td>
<td>14</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>0.5 (0.1–4.0)</td>
<td>1</td>
</tr>
<tr>
<td>03</td>
<td>25</td>
<td>21</td>
<td>22</td>
<td>0.8 (0.5–1.4)</td>
<td>13</td>
</tr>
<tr>
<td>04</td>
<td>28</td>
<td>19</td>
<td>20</td>
<td>0.6 (0.4–1.0)</td>
<td>15</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>20</td>
<td>21</td>
<td>1.6 (0.9–2.6)</td>
<td>7</td>
</tr>
<tr>
<td>12</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>1.3 (0.6–3.1)</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>28</td>
<td>21</td>
<td>22</td>
<td>0.7 (0.4–1.1)</td>
<td>15</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>5</td>
<td>5</td>
<td>0.9 (0.4–2.4)</td>
<td>3</td>
</tr>
<tr>
<td>07</td>
<td>19</td>
<td>12</td>
<td>12</td>
<td>0.6 (0.3–1.1)</td>
<td>10</td>
</tr>
<tr>
<td>08</td>
<td>5</td>
<td>1</td>
<td>1</td>
<td>0.2 (0.03–1.4)</td>
<td>3</td>
</tr>
<tr>
<td>09</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0.2 (0.01–0.3)</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>0.7 (0.2–2.3)</td>
<td>2</td>
</tr>
</tbody>
</table>

†$P = 0.0002$.
††$P = 0.001$.

HC, Healthy controls; UC, ulcerative colitis; n, number of individuals; N, number of alleles; AF, allele frequency; OR, odds ratio; CI, confidence interval.

### Table 2. Phenotype and haplotype frequencies of the five tumour necrosis factor (TNF) haplotypes in 98 UC patients and 98 controls

<table>
<thead>
<tr>
<th>TNF haplotype</th>
<th>HC n</th>
<th>%</th>
<th>UC n</th>
<th>%</th>
<th>UC versus control OR (95% CI)</th>
<th>HC N</th>
<th>%</th>
<th>UC N</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>25</td>
<td>26</td>
<td>35</td>
<td>36</td>
<td>1.6 (0.9–3.0)</td>
<td>27</td>
<td>14</td>
<td>39</td>
<td>20</td>
</tr>
<tr>
<td>E</td>
<td>33</td>
<td>34</td>
<td>26</td>
<td>27</td>
<td>0.7 (0.4–1.3)</td>
<td>37</td>
<td>19</td>
<td>30</td>
<td>15</td>
</tr>
<tr>
<td>H</td>
<td>10</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>0.5 (0.2–1.4)</td>
<td>10</td>
<td>5</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>I</td>
<td>34</td>
<td>35</td>
<td>39</td>
<td>40</td>
<td>1.2 (0.7–2.2)</td>
<td>40</td>
<td>20</td>
<td>43</td>
<td>22</td>
</tr>
<tr>
<td>P</td>
<td>65</td>
<td>66</td>
<td>61</td>
<td>62</td>
<td>0.8 (0.5–1.5)</td>
<td>82</td>
<td>42</td>
<td>79</td>
<td>40</td>
</tr>
</tbody>
</table>

HC, Healthy controls; UC, ulcerative colitis patients; n, number of individuals; OR, odds ratio; CI, confidence interval; N, number of haplotypes.

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one patient with stable proctitis was a carrier of this haplotype \( (P < 0.003; \text{OR} = 20.4; 95\% \text{CI} = 2.2–193) \).

The phenotype frequency of IL-1Ra allele 2 was 67% among patients with progressive disease, and was somewhat increased compared with patients with stable proctitis (43%), although the difference did not reach statistical significance.

**Need for operation.** Twenty-four patients (nine females, 15 males) underwent colectomy after a mean time after diagnosis of 7 years (range 0–27 years). The phenotype frequencies of DRB1*0103 in operated patients was 17%, and was significantly increased over controls \( (P < 0.001; \text{OR} = 84; 95\% \text{CI} = 9–785) \). In non-operated patients, the phenotype frequency was 3%, and did not differ significantly from the controls \( (P = 0.06; \text{OR} = 12; 95\% \text{CI} = 1–130) \).

Both DRB1*15 and IL-1Ra allele 2 were found more often in patients that underwent colectomy, but the \( P \) values did not retain significance when the number of comparisons was taken into account.

**Medication.** During the course of their disease, 53 patients were treated with systemic steroids, and 16 with immunosuppressive drugs (azathioprine or cyclosporin). Twenty-three patients were treated with neither glucocorticosteroids nor immunosuppressive drugs. No association was found between the need for these drugs and any of the markers studied.

**Family history and extra-intestinal manifestations.** In those patients with a positive family history, DRB1*15 was found in 50%, TNF-C in 40%, and allele 2 of the IL-1Ra in 60%, and did not differ with statistical significance from those without affected family members. Interestingly, none of the patients with a positive family history was DRB1*0103+.

Extra-intestinal manifestations were found in nine patients. This group was too small for statistical analysis. The three patients with skin manifestations, as well as the two patients with primary sclerosing cholangitis (PSC), were all DRB1*15+.

**DISCUSSION**

Much effort has already been made to delineate genomic regions containing genes involved in the disease process underlying IBD. Whole genome screening family-based linkage studies have recently revealed the first regions of interest in chromosome 16 for CD [2], and chromosomes 12, 7 and 3 for both UC and CD [3]. These data should be interpreted with great caution, as linkage studies investigate solely patients with multiple affected family members. As a positive family history is found in only 10–20% of patients, this group might actually represent a subgroup of patients, with a specific genetic susceptibility. As has recently been argued, in complex heterogeneous diseases like IBD, association studies in families have far greater power [30].

We have evaluated HLA-DR alleles as well as polymorphisms in the genes for TNF-\( \alpha \), LT-\( \alpha \), IL-1 and IL-1Ra in clinically well-defined patients, both at first visit to our hospital and at follow up. This novel approach introduces more certainty of disease susceptibility and behaviour of the disease. Using this approach, we have observed the following:

1. We confirm, for the first time, the association between DRB1*0103 and UC. This is of interest, as this allele is extremely rare in the Dutch population. Satsangi et al. observed that this allele was increased in their unselected population of UC patients from the UK [12]. Moreover, this allele was highly increased in patients with extensive colitis undergoing colectomy [20]. In agreement with these as well as preliminary results from the USA [31], we found that all our DRB1*0103+ patients had extensive disease, and four out of six patients had been colectomized at follow up. These findings support the observation that this allele may predict a more severe form of UC.
2. The DRB1*15 allele was found to be significantly associated with UC in general, and was strongly associated with female UC. The OR for UC in females was 3.5 compared with HC. We previously described this association in a limited number of Dutch UC patients [10]. This is the first report from Northern Europe showing a strong association between UC and DRB1*15 in a large group of Caucasians. To our knowledge, no other reports exist on HLA associations in UC and the female sex. This observation is of particular interest in view of recent findings in multiple sclerosis. In this disease, which has some remarkable clinical and immunogenetic similarities with UC, a significant association has been found between relapsing-remitting form of disease and HLA-DRB1*1501 in females, but not in males [32]. With regard to HLA-DR2 associations in UC, the different studies have had conflicting results. Studies from Japan and California (where a mixed Jewish/non-Jewish population was studied) have shown significant associations with DRB1*15+

As has been suggested, a possible explanation for the conflicting results might be that the DRB1*1502 is the responsible susceptibility allele, as this allele is the most frequent subspecificity of DRB1*15 in the Japanese and Jewish populations. In the Dutch population, DRB1*1501 is the most frequent allele, accounting for >95% of the DRB1*15 alleles. In our patient population, the large majority of DRB1*15+ individuals is DRB1*1501, which makes it unlikely that DRB1*1502 is the susceptibility allele for UC. We suggest that a shared epitope on the DRB1*15 molecule, or a gene in linkage with both DRB1*1501 and DRB1*1502, may determine the disease susceptibility.

On subgroup analysis, we found a very significant association between extensive colitis (in females) and DRB1*15, supporting previous observations from Japan [6] and the UK [20]. In the latter, no association between disease in general and this allele was found [12], but a significant association was found in patients undergoing colectomy, thus showing the importance of proper patient classification in genetic association studies.

Apart from the significant association between DRB1*0103 and DRB1*15 and extensive colitis, the TNF-C haplotype as well as the IL-1Ra allele 2 were predominantly found in those patients who developed extensive colitis. Moreover, both DRB1*0103 and DRB1*15 alleles and the IL-1Ra allele 2 were increased in those patients undergoing colectomy. However, most associations did not retain significance after correction for the number of comparisons. We could confirm our previous observation of a significant association between carriership of IL-1Ra allele 2 and the trend over the categories proctitis, left-sided colitis and pancolitis [15]. A striking finding was that in the group of proctitis patients >90% of carriers of the TNF-C haplotype had progression from proctitis to extensive colitis at follow up. Taken together, these findings support the hypothesis that multiple genes may be responsible for a more severe course of the disease, the HLA alleles being the most significant. However, based on the results from this study, we cannot conclude yet whether these genes act in conjunction or act separately in determining the severity of the disease.

There has been some interest in the relation between the presence of antibodies against the neutrophil granulocyte (p-ANCA) and DRB1*15. Yang et al. found that p-ANCA \(^+\) patients had a significantly increased prevalence of DR2 compared with ANCA \(^-\) controls [33]. We have studied p-ANCA for >5 years in a large group of patients, and have observed strong fluctuations in p-ANCA positivity at follow up. A considerable number of patients show seroconversion during their course of disease (unpublished results). However, in those patients that were consistently p-ANCA \(^+\) (22%), 47% were DRB1*15 \(^+\), suggesting that p-ANCA is not a subclinical marker for the DRB1*15 \(^+\) subgroup in our population.

One of the explanations for the association between several immune-mediated diseases and specific HLA alleles is that the HLA alleles are not directly involved in disease susceptibility but are markers for other closely linked genes. TNF-\(\alpha\) and LT-\(\alpha\) are cytokines with key functions in the immune response. Their genes are located in the central region of the MHC, between the class I and class II genes. In this regard it is of interest that several authors have shown a relation between the presence of HLA-DR2 and decreased production of TNF-\(\alpha\) [34,35]. The exact mechanism for this decreased secretion has not been unravelled yet. We previously studied TNF-\(\alpha\) secretion by peripheral blood mononuclear cells (PBMC) in relation to polymorphisms in the TNF genes, and found that the TNF-C haplotype is associated with decreased TNF-\(\alpha\) secretion, whereas the TNF-E haplotype is associated with high secretion of this cytokine [36]. These haplotypes differ only at position -308 in the promoter region of the TNFA gene. Recent results from another group indicate that this position is involved in the regulation of TNF-\(\alpha\) transcription [37]. These findings suggest that individuals carrying the DR2 allele or the TNF-C haplotype may be genetically predisposed to secrete low levels of TNF-\(\alpha\) upon an antigenic stimulus. Various studies have investigated whether TNF-\(\alpha\) secretion is altered in patients with UC. TNF-\(\alpha\) levels in UC patients have been measured in serum, in stools, in the mucosa of the gut, and in isolated PBMC. The results of these studies are highly inconsistent. Interestingly, two studies have shown a decreased secretion of TNF-\(\alpha\) in UC patients. Mazlam et al., when studying TNF-\(\alpha\) secretion by peripheral blood monocytes after stimulation with lipopolysaccharide (LPS), found a significantly decreased production of TNF-\(\alpha\) in UC patients compared with CD patients and HC [38]. We studied TNF-\(\alpha\) and LT-\(\alpha\) secretion by PBMC after T cell activation and found that the secretion of biologically active TNF was decreased in UC patients compared with HC [39]. In contrast to these findings, no studies exist on decreased TNF-\(\alpha\) secretion in CD. In the latter, several clinical and laboratory observations suggest a pivotal role for this cytokine in the pathogenesis of CD. Recent clinical studies using anti-TNF-\(\alpha\) MoAbs have underlined this [16,40,41]. In our study population, a large number of UC patients with extensive colitis is carrier of a low secretor TNF-\(\alpha\) genotype (either DRB1*15 or TNF-C). This may suggest that UC patients have a different immunological disturbance compared with CD patients, and may therefore not benefit to the same extent as CD patients from anti-TNF-\(\alpha\) treatment. Preliminary results from clinical trials support this concept, as this form of treatment was effective in only one of three studies [42–44]. Therefore, to unravel further the mechanism of action of this form of treatment, it will be of paramount importance to investigate the genetic background of these patients when evaluating this form of treatment in UC patients.

In conclusion, this study provides further evidence for the role of DRB1*0103 and DRB1*15 in disease susceptibility to UC. The DRB1*0103 and the DRB1*15 alleles (in females) predict a more aggressive form of disease, as they are associated with extensive colitis and an increased risk for colectomy. Neither the IL-1Ra nor the TNF genes are associated with overall disease susceptibility, but may play a modest role in determining the severity of the disease. The TNF-C haplotype may be a marker for progressive ulcerative colitis.

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