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Evidence for genetic heterogeneity in inflammatory bowel disease (IBD); HLA genes in the predisposition to suffer from ulcerative colitis (UC) and Crohn's disease (CD)

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SUMMARY

Family and epidemiological studies support a genetic susceptibility to UC and CD. Conflicting reports regarding associations between UC and HLA-DR2 and between CD and various HLA alleles have been published. The aim of this study was to determine whether molecularly defined HLA-DR genes are associated with these diseases in a Dutch group of patients. Fifty-nine unrelated Dutch UC patients and 89 CD patients were typed using DNA-based methods. A total of 2400 healthy local blood donors served as controls. The phenotype frequency of the HLA-DRB1*15 allele was increased in UC patients compared with controls (42% *versus* 26% in controls; $P = 0{\cdot}006$; odds ratio (OR) = 2·1), and was predominantly found in female patients (53% *versus* 24%; $P = 0{\cdot}001$; OR = 3·5). The DRB1*15 allele was increased in UC patients having a positive family history ($P = 0{\cdot}01$; OR = 5·8). Among the 16 patients who showed an increase in extent of disease during follow up, 10 were DRB1*15$^+$ ($P = 0{\cdot}002$; OR = 4·8). The frequency of the DRB1*13 allele was decreased in patients with UC (15% *versus* 28% in controls; $P = 0{\cdot}04$; OR = 0·5). In CD, no association was observed between disease or particular clinical subgroups and any allele tested. The present study provides additional evidence for the genetic association between UC and HLA-DRB1*15, and supports recent findings that the susceptibility gene(s) for CD is not located in the HLA class II region.

Keywords inflammatory bowel disease Crohn's disease ulcerative colitis HLA association heterogeneity

INTRODUCTION

The etiology of CD and UC has not been fully elucidated, but several observations support a genetic susceptibility to these chronic IBD [1,2]. These include an increased familial incidence [3,4], a higher prevalence of disease in monozygotic twins *versus* dizygotic twins [5,6], higher rates of disease in first degree relatives *versus* spouses [7,8], and differences in prevalence in distinct ethnic groups [2,9,10]. In spite of the fact that these observations strongly support the contribution of genetic factors in the pathogenesis of IBD, the relevant genes have not been identified. Multiple genes are probably involved and may operate at the level of the target organs or the immune response [11]. Recent studies using genome-wide screening in families with multiple IBD patients and concordant sib-pairs have confirmed the involvement of multiple genes in different chromosomes [12], although another group of investigators, using similar technology, found that only chromosome 16 was involved in susceptibility to CD [13]. Since the proteins of the MHC, in man called the Human Leucocyte Antigens (HLA), are essential for the regulation of the immune response, there have been various studies on associations between IBD and allelic variations of these genes. Although the results of these studies are not conclusive, several authors have reported an association between UC and HLA-DR2, especially in the Japanese population [14–18]. For CD, the associations are less clear. An association with HLA-DR1 was found by Toyoda *et al.* and was recently confirmed [17,19]. In this study from France, the authors also observed an increased prevalence of HLA-DR7, which was also found in a large study from Germany [19,20].

Recently, several linkage studies in families with IBD have been published, and yielded conflicting results. Satsangi *et al.* reported linkage of UC to the HLA-DRB1 locus [12,21], but this was not found in another family study from the UK [22]. Studies

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from France [13] and Oxford [21] regarding CD, however, provided strong evidence that the susceptibility for familial CD is located outside the HLA region.

In the present study, we investigated the distribution of the HLA alleles in Dutch patients with IBD. We also addressed the question whether HLA-DRB1 alleles are useful as a genetic marker for specific clinical subgroups of patients.

PATIENTS AND METHODS

Study population

UC patients. Fifty-nine unrelated Dutch Caucasian UC patients (30 females, 29 males) were included. 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 4 years; range 0–30 years), and at their latest visit (after a mean follow up of 10 years after diagnosis; range 1–32 years). Nine UC patients had a positive family history of IBD. The diagnosis UC or CD was based on conventional clinicopathological criteria as described by Lennard-Jones [23]. Mean age at diagnosis was 35 years (range 8–79 years). The localization of gut involvement was defined in UC as proctitis, left-sided (up to the splenic flexure), or pancolitis (beyond the splenic flexure), based on endoscopy and/or radiological investigations. At the time of diagnosis, 20 patients had distal disease, whereas 39 had extensive colitis (29 left-sided colitis and 10 pancolitis). At follow up, 11 patients had distal disease and 48 extensive disease (32 left-sided colitis and 17 pancolitis). Sixteen patients showed an increase in extent of disease during follow up (nine from proctitis to left-sided, six from left-sided to pancolitis and one from proctitis to pancolitis). At follow up, 15 patients had been operated.

CD patients. Eighty-nine unrelated Dutch Caucasian CD patients (62 females, 27 males) were included. Twelve individuals had a positive family history for IBD. Mean age at diagnosis was 26 years (range 10–60 years). Patients were referred to the Academic Hospital Vrije Universiteit after a mean time of 5 years (range 0–36 years) after diagnosis was made. Mean follow-up time was 7 years (range 0–29 years). Extension of disease in CD was defined as ileal, based on radiological findings, colonic, or combined small bowel and colonic disease, based on endoscopy and/or radiological investigations. At the time of diagnosis, 29 patients had colitis, 24 ileitis, and 36 had combined small bowel disease and colitis. At follow up, 23 patients had colitis, 17 had ileitis, whereas 47 had ileitis and colitis. Forty-two patients had been operated at follow up. Thirty-eight patients developed stenotic disease, whereas 30 had fistulas.

Control group. A group consisting of 2400 unrelated healthy Dutch Caucasian blood donors serologically typed for HLA served as controls. The ethnic background was comparable between the study and control groups. Part of this control group was also typed for the subtypes of 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.

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 [24] and biotin-labelled sequence-specific oligonucleotide probes (SSO) previously described [25].

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 χ^2 (with Yates' correction) or Fisher's exact test. We also tested the possibility of a 'dose–response' relationship across the categories non-carrier, heterozygous carrier, and homozygous carrier using the χ^2 test for trend.

RESULTS

HLA-DR in ulcerative colitis

The phenotype frequencies of the HLA-DR alleles in controls and in UC patients are given in Table 1. Gene frequencies are shown in Table 2. The phenotype frequency of the HLA-DRB1*15 (DRB1*15) allele was significantly increased in UC patients compared with controls (42% *versus* 26% in controls; $P = 0{\cdot}006$; odds ratio (OR) = 2·1; 95% confidence interval (CI) = 1·3–3·6). Focusing on DRB1*15, when comparing non-carriers, carriers and homozygous carriers, we found a significant trend across these categories (see Table 3; χ^2 for trend = 16·2; $P < 0{\cdot}001$).

The frequency of the DRB1*13 allele was decreased in UC patients (15% in UC patients *versus* 28% in controls; $P = 0{\cdot}04$; OR 0·5; 95% CI 0·2–0·9). When testing for trend across the categories non-carrier, heterozygous carrier, and homozygous carrier of DRB1*13, a significant negative association was observed (Table 3; χ^2 for trend = 5·4; $P = 0{\cdot}02$).

HLA-DR associations in Crohn's disease

The frequency distributions and gene frequencies of the DRB1 alleles in controls and in CD patients are given in Tables 1 and 2, respectively. No statistically significant association was observed between any HLA-DRB1 allele and CD. In particular, no significant association was found with DRB1*01 or DRB1*07.

HLA antigens in relation to clinical characteristics in UC patients

When patients were divided according to sex, we found that the DRB1*15 association observed was strongly associated with the female sex. In female UC patients, 16/30 were DRB1*15$^+$ (53% *versus* 24% in healthy controls (HC); $P = 0{\cdot}001$; OR 3·5; 95% CI 1·7–7·4; see Table 4). In male UC patients, 9/29 patients were carriers of this allele (31% *versus* 27%; $P = 0{\cdot}67$; OR 1·2; 95% CI 0·6–2·8).

Nine patients had a positive family history for IBD, among whom three had a first-degree relative with IBD. Six of nine patients were DRB1*15$^+$ ($P = 0{\cdot}01$; OR 5·8; 95% CI 1·4–23·2 compared with HC). The three patients with a first-degree relative with IBD were all DRB1*15$^+$.

The relation between disease localization and the DRB1*15 allele is given in Tables 5a and b. When compared with the control group, the frequency of DRB1*15 was significantly increased in those patients with extensive colitis (left-sided and pancolitis), both at time of diagnosis ($P = 0{\cdot}02$; OR 2·2; 95% CI 1·2–4·3), and at follow up ($P = 0{\cdot}001$; OR 2·7; 95% CI 1·5–4·7). When patients were divided according to sex, this association was found to be significant in female patients, both at time of diagnosis ($P < 0{\cdot}01$; OR 3·7; 95% CI 1·4–10), and at follow up ($P < 0{\cdot}0001$; OR 5·4;

Table 1. HLA-DR phenotype frequencies in 59 patients with UC, 89 patients with CD and 2400 controls

HLA-DRB1*	HC (%)	UC		CD		UC *versus* control OR (95% CI)	CD *versus* control OR (95% CI)
		(*n*)	(%)	(*n*)	(%)		
01	20	15	25	24	27	1·4 (0·8–2·6)	1·5 (0·96–2·5)
15	26	25	42	24	27	2·1 (1·3–3·6)†	1·1 (0·7–1·7)
16	2	1	2	0	0	0·9 (0·1–6·6)	0·3 (0·02–4·7)
03	25	11	19	20	22	0·7 (0·4–1·4)	0·9 (0·5–1·4)
04	28	13	22	21	24	0·7 (0·4–1·3)	0·8 (0·5–1·3)
11	14	12	20	16	18	1·5 (0·8–2·9)	1·3 (0·8–2·3)
12	5	4	7	4	4	1·5 (0·5–4·2)	1·0 (0·4–2·7)
13	28	9	15	26	29	0·5 (0·2–0·9)††	1·0 (0·7–1·7)
14	5	3	5	8	9	0·9 (0·3–3·0)	1·7 (0·8–3·6)
07	19	7	12	20	22	0·6 (0·3–1·3)	1·2 (0·7–2·0)
08	5	1	2	4	4	0·3 (0·04–2·2)	0·8 (0·3–2·3)
09	2	0	0	1	1	0·3 (0·02–5·6)	0·5 (0·06–3·4)
10	4	2	3	2	2	0·8 (0·2–3·3)	0·5 (0·1–2·2)

HC, Healthy controls; *n*, number of individuals; %, percentage of individuals; OR, odds ratio; CI, confidence interval.
† $P = 0{\cdot}006$; OR = 2·1; 95% CI = 1·3–3·6.
†† $P = 0{\cdot}04$; OR = 0·5; 95% CI = 0·2–0·9.

95% CI 2·3–13), but not in male UC patients ($P = 0{\cdot}5$; OR 1·3; 95% CI 0·5–3·3 and $P = 0{\cdot}5$; OR 1·4; 95% CI 0·6–3·2, respectively). No statistically significant difference in the phenotype frequency of DRB1*15 was observed between patients with proctitis and controls, neither at the time of diagnosis ($P = 0{\cdot}2$; OR 1·9; 95% CI 0·8–4·8), nor at follow up ($P = 0{\cdot}7$; OR 0·6; 95% CI 0·1–3).

Sixteen patients showed an increase in extent of disease during follow up (nine from proctitis to left-sided, six from left-sided to pancolitis, and one from proctitis to pancolitis). The mean follow-up time in this group was higher than in those patients who did not have an increase in extent of disease (14 *versus* 9 years, $P = 0{\cdot}01$). Ten of these patients were DRB1*15^{+} ($P = 0{\cdot}002$; OR 4·8; 95% CI 1·7–13·3 compared with HC). In this subgroup, the follow-up time did not differ between carriers of this allele and non-carriers (14 *versus* 15 years).

No association was found with any HLA-DR allele when patients were subdivided according to age at onset of disease.

At follow up, 15 patients had been operated. No statistically significant association was found between the need for operation and the DRB1*15 allele, as six patients were carriers of this allele ($P = 0{\cdot}2$; OR 1·9; 95% CI 0·7–5·5 compared with controls). No association with any other allele was found, either.

HLA antigens in relation to clinical characteristics in CD patients
No statistically significant associations were found with any HLA-DR allele when patients were divided according to positive family history, sex, age of onset, the need for operation, or disease type (stenotic or fibrotic). Furthermore, no association was found between localization of disease and any HLA-DR allele tested.

Table 2. HLA-DR allele frequencies in 59 UC patients, 89 CD patients and 2400 healthy controls (HC)

HLA-DRB1*	HC (%)	UC		CD	
		(*n*)	(%)	(*n*)	(%)
01	10	18	15	25	14
15	14	31	26	25	14
16	1	1	1	0	0
03	13	13	11	22	12
04	15	14	12	21	12
11	7	14	12	16	9
12	2	4	3	4	2
13	15	9	8	28	16
14	3	4	3	8	4
07	10	7	6	22	12
08	3	1	1	4	2
09	1	0	0	1	1
10	2	2	2	2	1

n, Number of alleles; %, percentage of alleles.

DISCUSSION

There is ample evidence to support the necessity of a genetic predisposition to IBD [1,2]. Thus, genetic studies are essential to establish the biological basis of this predisposition. The recent studies performed in families with multiple affected cases provide further evidence for the existence of a genetic predisposition, although due to the relatively small number of families studied no firm conclusions can be drawn as yet [12,13]. In the study from Oxford it was found that in UC, the sharing of alleles among affected sibling pairs provided evidence for linkage with the DRB1 locus, but no specific association with the DRB1*15 allele was found [12]. Association studies in some populations have found, however, that UC is associated with the DRB1*15 allele. This association was found to be significant in the Japanese population (DRB1*1502) and in some groups of patients reported in the USA and the UK (DRB1*1501) [15–17,26,27]. In other studies the difference did not reach statistical significance [28,29]. Finally, in some studies a decreased frequency of HLA-DR2 was observed [30,31]. The studies from Japan and the USA are of interest

Table 3. Distribution of homozygous and heterozygous carriers of HLA-DRB1*15 and HLA-DRB1*13 in UC and controls

	Homozygous carriers (%)	Heterozygous carriers (%)	Non-carriers (%)
	DRB1*15	DRB1*15	DRB1*15
UC patients ($n = 59$)	7 (12)	18 (31)	34 (58)
Controls ($n = 1576$)	30 (2)	375 (24)	1171 (74)†
	DRB1*13	DRB1*13	DRB1*13
UC patients ($n = 59$)	0 (0)	9 (15)	50 (85)
Controls ($n = 2355$)	56 (2)	612 (26)	1687 (72)††

n, Number of individuals.
† χ^2 for trend = 16·2; $P < 0{\cdot}001$.
†† χ^2 for trend = 5·4; $P = 0{\cdot}02$.

because of the more homogeneous population (Japanese and Jewish), which would diminish genetic heterogeneity. This offers a plausible explanation for the conflicting and inconclusive results from studies in the Caucasian population. A carefully selected ethnically matched control group and maximum sample size should help to obtain adequate results. In our study, an increased number of patients with UC carried the HLA-DR2 (DRB1*15) antigen, and this allele was predominantly found in female patients. The phenotype frequency of this allele was similar to the percentage observed in the USA by Toyoda *et al.* [17]. Interestingly, in both studies a decreased frequency of HLA-DR6 was found.

Evidence is emerging suggesting that several genes in the HLA region may play a role in the severity and course of IBD. It has been noted that the association between HLA-DR2 and UC appears to be stronger in patients with extensive disease [15]. Furthermore, it was found that in Japanese UC patients the allele DRB1*1502 is associated with the need for steroid treatment [16]. These results support the concept that genes in the HLA region may contribute to severity of the disease and therefore have a role in prognosis. In the present study we also investigated the prevalence of the HLA-DR alleles in relation to clinical characteristics. As the course of disease may fluctuate at different time points, we studied the HLA-associations at two time points: when patients first visited our hospital, and at their latest visit. Using this approach, we found a high prevalence of DRB1*15 in those UC patients who showed an increase in their extent of bowel involvement at follow up. We also observed that the DRB1*15 allele was significantly associated with extensive colitis, but not with proctitis. Furthermore, we observed a high prevalence of DRB1*15 in patients with a positive family history for IBD. Although our study population was small, these observations together support the concept that the DRB1*15 allele may be a marker for specific subgroups of UC.

We found no association between CD and any HLA-DR allele tested. These findings are in agreement with older studies from The Netherlands [32] and UK [33]. Recent linkage studies from France [13] and the UK [21] have provided strong evidence that, at least in familial CD, the susceptibility genes are located outside the HLA region.

The reason for the association between several diseases and specific HLA alleles is largely unknown. It is even questionable whether these genes are responsible for the predisposition to the disease rather than markers for other closely linked genes. The association observed might, in fact, be due to the strong linkage disequilibrium with other class II genes. Therefore, studies on the

Table 4. Frequency of HLA-DRB1*15 in female patients with UC and controls

	UC females (%)	Healthy females (%)
DRB1*15$^+$	16 (53)	192 (24)
DRB1*15$^-$	14 (47)	593 (76)

$P = 0{\cdot}001$; OR = 3·5; 95% CI = 1·7–7·4.

Table 5. a. Distribution of the HLA-DRB1*15 allele in UC patients with proctitis, left-sided colitis and pancolitis at time of diagnosis

	Proctitis			Extensive colitis		
	Females	Males	Total	Females	Males	Total
DRB1*15$^+$ (*n*)	7	1	8	9†	8	17††
DRB1*15$^-$ (*n*)	7	5	12	7	15	22

n, Number of patients.
† $P < 0{\cdot}01$; OR = 3·7; 95% CI = 1·4–10 (extensive colitis *versus* controls).
†† $P = 0{\cdot}02$; OR = 2·2; 95% CI = 1·2–4·3 (extensive colitis *versus* controls).

b. Distribution of the HLA-DRB1*15 allele in UC patients with proctitis, left-sided colitis and pancolitis at follow up

	Proctitis			Extensive colitis		
	Females	Males	Total	Females	Males	Total
DRB1*15$^+$ (*n*)	1	1	2	15†	8	23††
DRB1*15$^-$ (*n*)	6	3	9	8	17	25

n, Number of patients.
† $P < 0{\cdot}0001$; OR = 5·4; 95% CI = 2·3–13 (extensive colitis *versus* controls).
†† $P = 0{\cdot}001$; OR = 2·7; 95% CI = 1·5–4·7 (extensive colitis *versus* controls).

HLA-DQ genes are ongoing. Furthermore, several immunologically important genes are located within the MHC, such as non-HLA genes for heat shock proteins, complement factors and the genes for tumour necrosis factor-alpha (TNF-α) and lymphotoxin-alpha (LT-α). We previously studied polymorphisms in the TNF-α and LT-α genes that are in linkage disequilibrium with the HLA class II genes [34]. A statistically significant association between a biallelic polymorphism at position -308 in the promoter region of the TNF-α gene and UC was found. Future family studies and studies in different ethnic groups are necessary to investigate whether this allele and DRB1*15 are independently associated with the susceptibility to develop UC.

In conclusion, the present study suggests that genes within the HLA system play a modest role in susceptibility to UC. The possibility that carrying a particular allele such as the HLA-DR2 has an effect on prognosis is interesting, and further long-term follow-up studies are indicated. Even in populations where the association with HLA-DR2 is not significant, the presence of this allele may indicate that these patients will develop total colonic involvement in the course of years. We believe that nowadays it is less important to determine whether particular alleles are associated with the disease in general. Prospective genetic studies of clinically well defined patients may give new clues to understand the pathogenesis and disease heterogeneity of these diseases.

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