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HLA-DRB1*1502 confers susceptibility to ulcerative colitis, but is negatively associated with its intractability: a Korean study

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Abstract *Background and aims:* Several studies have documented the high incidence of several HLA class II alleles in Japanese patients with ulcerative colitis (UC). Although the characteristics of the HLA system in Koreans are quite similar to those in the Japanese, it is not clear whether the HLA pattern in Korean UC is similar to that in Japanese UC. We investigated an association between HLA class II genes and UC patients and the clinical meaning of these genes in Korea. *Patients and methods:* Unrelated Korean patients with UC (*n*=70) and ethnically matched unrelated controls (*n*=182) were genotyped for HLA-DR by PCR followed by reverse hybridization using sequence-specific oligonucleotide probes. The clinical characteristics of the patients were analyzed with regard to anti-neutrophil cytoplasmic antibody (ANCA) status and total colectomy for intractability. *Results:* HLA-DR2 and DRB1*1502 were found significantly more frequently in patients (42.9% and 21.4%) than controls (20.3% and 5.5%). DRB1*1502 was more frequent in p-ANCA-positive (5/23) than in p-ANCA-negative (1/11) patients. Total colectomy for intractability was performed more commonly in patients without DRB1*1502 (14/55) than in those with it (0/15). *Conclusions:* Our data are consistent with those of Japanese studies in that DR2 and DRB1*1502 are positively associated with UC patients. In contrast to the Japanese study, however, our results demonstrates that DRB1*1502 is negatively associated with the risk of colectomy in Korean patients with UC.

Keywords Ulcerative colitis · HLA · Genetics · Anti-neutrophil cytoplasmic antibody

Introduction

The idea that genetic factors have a role in the pathogenesis of ulcerative colitis (UC) has been supported by several kinds of studies demonstrating familial aggregation, increased monozygotic twin concordance, lack of increased risk in spouses, and ethnic aggregation [1]. Several genome wide scans and numerous replication studies have been uniquely successful in inflammatory bowel disease [2, 3]. Therefore, significant areas of replicated linkage have been found on chromosomes 6p (*IBD3*), 12q (*IBD2*), 14q, and 16q (*IBD1*).

Among these genes, highly polymorphic genes, such as HLA class II genes on chromosome 6, have been implicated as candidates for conferring the genetic susceptibility to UC [4, 5, 6, 7]. HLA-DR2, especially the HLA-DRB1*1502 allele, is reported to have a positive association with UC in Japanese patients [4, 8, 9]. Moreover, the HLA-DRB1*1502 allele is claimed to have an association with more extensive and/or refractory disease in some Japanese studies [10, 11]. However, data regarding an HLA-DR2 association in non-Japanese populations have shown conflicting results [12, 13, 14]. Most of non-Japanese studies have been performed in whites,

and ethnic matching was with Asian populations other than the Japanese. It has been postulated that population heterogeneity, in addition to small sample sizes, inadequate phenotyping methods, and/or disease heterogeneity within UC contribute to these conflicting results [4, 7]. Moreover, other alleles such as HLA-DR4 [4, 8] and HLA-DRB1*0103 [7] are also reported to be associated with UC.

Koreans have very high genetic homogeneity, which makes it easy to include ethnically matched cases and controls [15, 16]. Although the characteristics of the HLA system in Koreans are quite similar to those in the Japanese, there are some clear differences in the system between the two populations. For example, HLA-DR2 is less frequent in Koreans than in the Japanese [15]. Investigation of the HLA-DR association with UC in Koreans may thus contribute to our knowledge about the role of HLA-DR in the pathogenesis of UC. The present study investigated whether HLA-DR is positively associated with UC in Korean patients, and whether there is any clinical significance of these genetic alleles, including intractability and p-ANCA status.

Materials and methods

Study population

Seventy unrelated Korean patients with a definite diagnosis of UC and ethnically matched unrelated healthy Korean controls (n=182) were included in the present study for HLA-DR genotyping. The diagnosis of UC was based on conventional clinical and pathological criteria described previously. Clinical assessment and p-ANCA evaluations were performed before HLA-DR was genotyped. The clinical data collected included sex, age at onset of symptoms, age at diagnosis, disease extent, and need for surgery. The male to female ratio was quite similar between the patient group (34:36) and the control group (92:90). The age at the onset of disease ranged from 10 to 61 years, with a mean of 39.3 years.

Extent of disease

Extent of disease was determined by both total colonoscopy and microscopic examination in all cases. Based on the extent of involvement the patients were classified into three groups, including: (a) proctitis (inflammation confined to the rectum), (b) left-sided colitis (inflammation up to the splenic flexure), and (c) extensive colitis (inflammation beyond the splenic flexure).

Need for surgery

Of the 70 patients, 14 underwent total protocolectomy for intractability to medical therapy 3–48 months (median 25) after the diagnosis. The median follow-up duration of the unoperated patients was 42 months (7–120; P<0.05 vs. operated patients).

ANCA assay

p-ANCA was detected using the method recommended by the first International Workshop on ANCA, with minor modifications [17]. Briefly, cytocentrifuged smears with approximately 150,000 neutrophils per slide were fixed in absolute ethanol for 5 min at 4°C. Slides were then incubated with 1:20 diluted sera from patients as well as positive and negative controls, stained with fluorescein isothiocyanate conjugated rabbit anti-human IgG $F(ab')_2$ fragment (Dakopatts, Copenhagen, Denmark), and evaluated by fluorescence microscopy. Serum samples giving a typical perinuclear fluorescence at a dilution of 1:20 were regarded as positive.

HLA-DR genotyping

Molecular DNA-based HLA-DR typing was performed using a commercially available system, the Inno-LiPA HLA-DRB kit (Innogenetics, Gent, Belgium), based on the polymerase chain reaction amplification of HLA-DR sequences followed by reverse dot–blot hybridization with sequence-specific oligonucleotide probes [18]. Briefly, genomic DNA was prepared from peripheral blood leukocytes as described previously. HLA-DRB loci were amplified using biotinylated primers supplied by Innogenetics. The biotinylated polymerase chain reaction products were then hybridized to 31 sequence-specific oligonucleotide probes spotted on two Inno-Lipa strips. After color development with alkaline phosphatase conjugate and nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate, the hybridization pattern was interpreted with an Inno-Lipa Expert genotyping program V3.0 provided by Innogenetics.

The system can discriminate over 80 DRB1 alleles including DRB1*1502 and DRB1*0103, which were our main interest.

Statistical analysis

Statistical analysis of HLA-DR allele frequencies between the patient group and the control group or of any association of the alleles with subsets of UC was performed using the χ^2 test or Fisher's exact test depending on the sample size. A difference was regarded as significant when the P value was less than 0.05.

Results

There were 14 patients with proctitis, 30 with left-sided colitis, and 26 with extensive colitis. Table 1 shows the results of HLA-DR2, DRB1*1502, DR4, and DRB1*0103 typing in controls and patients with UC. HLA-DR2 was found to be significantly more frequent in UC patients than in controls (42.9% vs. 20.3%, P<0.01). Moreover, the frequency of DRB1*1502 was significantly higher in UC than in controls (21.4% vs. 5.5%, P<0.01). However, the frequency of DR4 and DRB1*0103 was not different

Table 1 Association of HLA-DR and ulcerative colitis

	Ulcerative colitis (n=70)		Control (n=182)		P
	n	%	n	%	
HLA-DR2	30	42.9	37	20.3	0.001
DRB1*1502	15	21.4	10	5.5	0.0005
HLA-DR4	19	27.1	67	36.8	NS
DRB1*0103	0	–	0	–	NS

Table 2 HLA-DR allele frequencies in UC and controls according to p-ANCA status

	Ulcerative colitis							
	p-ANCA positive (*n*=48)		p-ANCA negative (*n*=22)		Total (*n*=70)		Control (*n*=182)	
	n	%	*n*	%	*n*	%	*n*	%
HLA-DR2	17	35.4	13	59.1	30	42.9	37	20.3
DRB1*1502	10	20.8	5	22.7	15	21.4	10	5.5
HLA-DR4	13	27.1	6	27.3	19	27.1	67	36.8
DRB1*0103	0	–	0	–	0	–	0	–

between UC and controls. Clinical features including onset of disease and disease extent did not differ according to HLA-DR status.

The UC patients were then stratified into two groups on the basis of p-ANCA status. The frequencies of DRB1*1502, DR2, DR4, and DRB1*0103 were similar between the p-ANCA positive and p-ANCA negative groups (Table 2). To investigate the association of HLA-DRB1*1502 with clinical intractability, the clinical course of the patients was reviewed. Total colectomy for intractability was performed more commonly in patients without DRB1*1502 (14/55) than in those with it (0/15; $P<0.05$).

Discussion

It has been suggested that susceptibility to inflammatory bowel disease (IBD) is partially genetically determined [1]. Several genome-wide scans and numerous replication studies have been successful in IBD, and the data from these studies have been remarkably consistent [2, 3]. Therefore significant areas of replicated linkage have been found on chromosomes 6p (*IBD3*), 12q (*IBD2*), 14q, and 16q (*IBD1*). Among them, the HLA region, located on the short arm of chromosome 6, has been the most actively studied candidate gene region [4, 5, 6, 7]. This region contains a number of genes important in the immune response. Some of these genes determine the specificity of immune response. Also, this region includes genes that determine the level of the immune response, such as tumor necrosis factor (TNF) [19]. Cytokine genes such as those of TNFα and interleukins are also sought for the possible association of polymorphism and predisposition to the disease [19].

HLA class II genes have become representative candidates for a role in the pathogenesis of IBD as their products play a central role in the immune process. Therefore, there has been intensive research about the HLA II genes and IBD [4, 5, 6, 7]. However, the association of HLA class II genes with either Crohn's disease or UC has shown very different results between white and Japanese populations [8, 9, 12, 13, 14]. Moreover, the frequency of HLA class II alleles differs markedly between Jews (Israel), North European non-Jews, and Japanese. For instances, in the white non-Jewish population, DRB*1501 is the only common allele of DR2 and DRB1*1502 accounts for fewer than 5% of alleles. In contrast, DRB1*1502 is the most common DR2 allele in the Japanese and Jewish populations [13]. Thus, it is necessary to analyze the association of HLA class II alleles with IBD in a homogeneous population.

Koreans have very high genetic homogeneity. Based on the HLA haplotype analysis the Korean population is considered to be closest to the Japanese population [15, 16]. The antigen HLA-Bw54, previously thought to be unique to the Japanese (af=15%) and designated "J-1" by Julit et al. [20] has also been observed in Koreans (af=11%). However, in comparative study of HLA typing, Koreans have distinct feature in HLA class II. For example, the frequency of DRw6, an antigen not observed in Chinese was higher in Koreans than either Japanese or Caucasians. HLA-DR4, DR5, and DR7 were found more frequently in Koreans than Japanese or Chinese, while DR2 was less frequent in Koreans than in Japanese or Chinese. HLA-DR 1 and DRw8 were more frequent in Japanese than in Koreans, and DR3 was more frequent in Chinese than in Koreans. HLA-DQw3 was observed more frequently in Koreans than in either Japanese or Chinese [15]. Hence, Koreans are a suitable source of controls and patients strictly matched for ethnicity since Koreans comprise a very homogeneous and distinctive population.

Among class II molecules of HLA, many alleles have been studied in IBD. However, different studies report different results. The association of DR2 with UC has frequently been noted in the homogeneous Japanese population [8, 9, 23, 24, 25, 26]. However, studies in more heterogeneous white populations yield conflicting results. Some studies have found increased [12], equal [13], or even lower [14] frequencies. Our data from the homogeneous Korean population show that DR2 is associated with UC. A recent meta-analysis also confirms the association of DR2 with UC even when the Japanese studies are left out of the analysis, suggesting that DR2 is firmly associated with UC [4]. These results may be

either a population-specific effect based on linkage disequilibrium with the real 6p risk mutation or a genuine causal effect of the reported HLA alleles in UC. Further studies are needed to elucidate these association.

Moreover, we tested HLA-DRB1*1502 in our populations. Several reports have found an association between DRB1*1502 and UC [10, 11]. However, reports associating intractability and HLA are rare. Masuda et al. [10] reported that DRB1*1502 or DRw11 has a probability of being closely related to the intractability of UC. Others have reported that the DRB1*15 allele is significantly increased and is found predominantly in patients with extensive colitis [11]. In our data, however, DRB1*1502 was negatively related to clinical intractability in UC. It is difficult to explain why these contradictory results were obtained from genetically similar populations. Also, these results may involve the purportedly similar but separate genetic backgrounds of Koreans and Japanese. Also, it should be further elucidated how this distinct association of the HLA gene with the intractability of UC occurs. Some authors suggest that the association is due to the relationship between HLA and cytokine expression, such as interferon-γ and interleukin-4 [10]. However, further data are needed to elucidate the pathogenetic mechanism. A recent meta-analysis found DR4 and DRB1*0103 to have a negative and positive association with UC, respectively [4]. However, we did not find this association in our population.

ANCA has been reported to be associated with UC since 1990 [27]. The prevalence of positive p-ANCA in patients with UC ranges from 50% to 86% [28, 29, 30]. As there is a large amount of evidence suggesting genetic heterogeneity within IBD, there have been many investigations to subdivide these disorders into more etiologically homogeneous groups on the basis of both physiological defects and genetic marker associations. Yang et al. [31] studied two subgroups of UC by HLA typing and p-ANCA status. The p-ANCA positive group was associated with HLA-DR2, whereas the p-ANCA negative group was associated with HLA-DR4. However, a subsequent study found no association of HLA-DR2 with a p-ANCA positive subgroup of UC [32]. In Asians another allele, HLA-DQα1c, has been reported to be associated with p-ANCA positivity [33]. These conflicting results regarding an association between p-ANCA and HLA also suggest that the conclusion should be made according to ethnic difference. Our study observed no association between p-ANCA status and HLA types. Therefore in our population, it is uncertain whether there are some genetically distinct subgroups of UC patients according to p-ANCA positivity.

Conclusion

We observed the association of HLA-DR2 and HLA-DRB1*1502 with UC in ethnically distinct Korean patients. Moreover, HLA-DRB1*1502 was negatively related to intractability of disease. These data suggest that genetic factors play a role in the development of UC in Korean patients. These data may provide a basic source of information for further work about the clinical application of IBD genetic research, including molecular classification of disease or pharmacogenomics.

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