Association of HLA class II genes with susceptibility to Crohn's disease

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Abstract

Background—Published studies on the association between HLA class II genes and inflammatory bowel disease are contradictory perhaps because of the limited size and ethnic heterogeneity of the populations studied.

Aim—To compare the frequencies of HLA class II genes in a large number of French patients with Crohn's disease and in an ethnically matched control group.

Methods—344 patients (196 F, 148 M, mean age 23·6 years) with Crohn's disease were molecularly genotyped for the HLA-DQB1 and DRB1 alleles. The results were compared with those for an ethnically matched control population of 488 white adults.

Results—There were two significant variations of alleles at the DQB1 locus: an increase in DQB1*0501 allele frequency ($\chi^2=10\cdot6$, corrected p value (pc)=0·01, odds ratio (OR)=1·61) and a decrease in DQB1*0602/0603 allele frequencies ($\chi^2=8\cdot43$, pc=0·037, OR=0·66). DRB1 analysis showed associations with three allelic variations: an increase in the frequencies of DRB1*01 ($\chi^2=12\cdot86$, pc=0·003, OR=1·75) and DRB1*07 alleles ($\chi^2=11\cdot18$, pc=0·008, OR=1·58) and a very significant decrease in that of the DRB1*03 allele ($\chi^2=19\cdot7$, pc=$9\cdot10^{-5}$, OR=0·46).

Conclusion—The alleles DRB1*01 and DRB1*07 are associated with susceptibility to Crohn's disease. The strong negative association between the DRB1*03 allele and Crohn's disease suggests that the HLA-DRB1*03 allele mediates 'resistance' to Crohn's disease.

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Inflammatory bowel diseases (IBD) are complex, multifactorial diseases partly determined by genetic predisposition.[1] Particular attention has been paid to genes related to immune function, as the prevailing view is that an immunological process participates in IBD.[1] A linkage analysis of chromosome 6 loci in familial aggregations of Crohn's disease (CD) showed neither individual nor combined lod scores for any family or any locus high enough to suggest linkage or genetic heterogeneity.[2] However, this finding does not exclude the possibility that HLA genes might modulate the expression of the disease. Studies of the association of IBD with HLA class II genes may still be important because HLA genes restrict antigen recognition, control the immune response, and are associated with autoimmune disorders. Most association studies carried out so far have used serological techniques. Seven studies have shown an increase in the frequency of HLA-DR2 in ulcerative colitis (UC), although only two reached statistical significance.[1 3] Results in CD are even more controversial. Japanese workers found a positive association between DR4 antigen and CD,[4] whereas Smolen et al[5] reported a slight increase in HLA-DR7 antigen. The difference in HLA-DR7 antigen between CD and UC was highly significant. These conflicting results may result from the limited size and ethnical heterogeneity of the populations studied and the use of serological techniques, which did not discriminate well enough between HLA class II specificities. Few studies have examined the role of HLA genes as immunogenetic markers of IBD using novel genotyping procedures. Recently, Toyoda et al[6] described a positive association of UC with DR2 alleles and of CD with the combination of DR1 and DQ5 alleles in a Californian population. But other reports have failed to confirm these associations in European populations.[7 8]

This report describes the analysis of HLA class II genes in a large number of French patients; there were positive associations between CD and DRB1*01 and DRB1*07 alleles and a strong negative association between CD and DRB1*03 alleles.

Methods

Crohn's disease

A total of 344 unrelated white patients with CD were studied (196 women and 148 men, mean age 23·6 years, range 3–74). CD was diagnosed using previously described criteria.[9] All patients were from the north of France (48%, 135 adults, 30 children), Paris region (30%, 56 adults, 46 children) or Brittany (22%, 67 adults, 10 children).

Control population

Healthy white subjects were selected at random from each region and their HLA DQB1 and DRB1 alleles were genotyped. In all, 488 subjects were analysed (44% from the north of France, 38% from Brittany, and 18% from Paris). The control results were pooled, as no significant differences in allele

TABLE I   *Frequencies of HLA-DQB1 alleles in patients with Crohn's disease (CD) and in controls*

DQB1* allele	Control group (n=976)		CD patients (n=686)*		CD versus controls		
	n	%	n	%	pc	χ^2	OR (CI)
0201	205	21·00	140	20·41	NS		
0301	192	19·67	157	22·89	NS		
0302	100	10·25	53	7·73	NS		
0303	44	4·51	30	4·37	NS		
0304/0305	3	0·31	2	0·29	NS		
0401/0402	27	2·77	17	2·48	NS		
0501	*100*	*10·25*	*107*	*15·60*	*0·01*	*10·6*	*1·61 (1·21 to 2·17)*
0502	19	1·95	9	1·31	NS		
0503	42	4·30	26	3·79	NS		
0601	10	1·02	6	0·87	NS		
0602/0603	*185*	*18·95*	*93*	*13·56*	*0·037*	*8·43*	*0·66 (0·51 to 0·88)*
0604/0605	49	5·02	46	6·70	NS		

n: Number of alleles; pc: p corrected values; OR: odds ratio; (CI): confidence interval; *: 1 of 344 patients was not genotyped.

TABLE II   *Frequencies of HLA-DRB1 alleles in patients with Crohn's disease (CD) and in controls*

DRB1* allele	Control group (n=976)		CD patients (n=688)		CD versus controls		
	n	%	n	%	pc	χ^2	OR (CI)
01	*88*	*9·02*	*101*	*14·68*	*0·003*	*12·86*	*1·75 (1·28 to 2·35)*
15/16	135	13·83	77	11·19	NS		
03	*130*	*13·32*	*45*	*6·54*	*9·10⁻⁵*	*19·7*	*0·46 (0·31 to 0·65)*
04	165	16·91	105	15·26	NS		
11/12	129	13·22	97	14·10	NS		
13/14	175	17·93	117	17·01	NS		
07	*109*	*11·17*	*116*	*16·86*	*0·008*	*11·18*	*1·58 (1·21 to 2·13)*
08	27	2·77	19	2·76	NS		
09	9	0·92	4	0·58	NS		
10	9	0·92	7	1·02	NS		

Abbreviations as in Table I.

frequencies between the three regions were observed.

HLA class II genotyping

DNA was extracted from fresh or frozen peripheral blood leucocytes by standard techniques; genomic DNA was isolated by phenol-chloroform extraction of dodecyl-sulphate-lysed and proteinase K treated cells. The second exon encoding the polymorphic outer domains of HLA DRβ and DQβ chains was amplified separately by a polymerase chain reaction (PCR) procedure using specific flanking primers.[10] The DRB1 exon 2 was amplified using a procedure initially described by Yunis et al[11] and called amplification fragment length polymorphism (AFLP).[12] Six group specific sense primers and one antisense primer were used for the amplification of HLA-DRB1 alleles. DQB1 alleles were also typed using appropriate primers.[13] The results obtained with this technique and other molecular methods are in good agreement.[14 15]

After amplification, 10 µl of PCR products were taken for each digestion reaction. Different panels of restriction endonucleases were used according to the locus studied. Digested DNA samples were then run on an 8% w/v polyacrylamide vertical electrophoresis gel (200 V, 300 mA) and fragments were visualised by staining with ethidium bromide. Restriction endonuclease mapping of the PCR products provided a simple and precise way of defining HLA class II alleles at the nucleotide level. The nomenclature for factors of the HLA system 1994 was used throughout.[16]

Statistical analysis

The statistical significance of differences in allele frequencies between patients and controls was measured by the χ^2 test with Yates's correction. The level of significance was $p<0·05$. For allelic comparisons, p values were corrected (pc) for the number of comparisons according to Svejgaard et al.[17] For correlations between HLA alleles and clinical sub-groups, p values were corrected for the number of alleles studied and by the number of different sub-groups.

Results

Frequency of HLA alleles

Tables I and II give the comparisons of allele frequencies in CD patients and controls. The corrected p values indicated two significant variations in the DQB1 locus: an increase in DQB1*0501 allele frequency ($\chi^2=10·6$, pc= 0·01, odds ratio (OR)=1·61), and a decrease in the frequencies of the DQB1*0602/0603 alleles ($\chi^2=8·43$, pc=0·037, OR=0·66). DRB1 analysis showed associations with three allelic variations: an increase in the frequencies of HLA-DRB1*01 ($\chi^2=12·86$, pc=0·003, OR=1·75) and HLA-DRB1*07 alleles ($\chi^2= 11·18$, pc=0·008, OR=1·58) and a very significant decrease in that of the HLA-DRB1*03 allele ($\chi^2=19·7$, pc=9·10⁻⁵, OR= 0·46).

HLA-DRB1-DQB1 allele combinations

DRB1-DQB1 haplotypes were assigned on the basis of two locus associations and known linkage disequilibria, as described in the 11th International Histocompatibility Workshop.[18] HLA-DRB1*01 alleles are in strong linkage disequilibrium with the DQB1*0501 allele in white populations. The combination DRB1*01-DQB1*0501 was overexpressed in the CD population, but neither the p value nor OR were greater than those for DRB1*01 or DQB1*0501 alone (data not shown).

DRB1*1301, DRB1*1302, and DRB1*1401 were in linkage disequilibrium with DQB1* 0603, DQB1*0604, and DQB1*05031 respectively. The most important alleles in DRB1*02 are DRB1*1501 and DRB1*1601, which were in linkage disequilibrium with DQB1*0602 and DQB1*0502 respectively in the French population (data not shown). As the frequencies of these two DRB1 alleles were not changed in CD, the significant decrease in the frequencies of the DQB1*0602 and *0603 alleles seems to be allelic.

No haplotypic effect was observed for DRB1*03 or DRB1*07 alleles.

HLA alleles and clinical features

On sub-group analysis no association between allelic frequency, and groups defined by sex, age at diagnosis, disease location, disease type,[19] previous surgery, and the use of immunosuppressive was found.

Discussion

Using HLA genotyping procedures, we showed an overall positive association between CD and DRB1*01 and DRB1*07 alleles and a negative association between CD and the DRB1*03 allele. These results are probably relevant because we studied a large population taken from three different regions of France, and obtained homogeneous results in both the patient group and controls. Furthermore, the statistical testing of allele distribution was done with correction for the number of antigens tested, which makes the results more powerful.

HLA-DRB1*03 is positively associated with several diseases, including type I diabetes, primary sclerosing cholangitis, and coeliac disease.[20][21] Conversely, the most impressive result that we observed in this study was an important decrease in HLA-DRB1*03 in CD. The estimated strength of the negative association between carrying these alleles and CD was OR50·46. This variation has also been described in a preliminary report from Germany.[8] It might even be a wider characteristic of IBD, as we have also observed a negative association with DRB1*03 in patients with UC.[22] If confirmed, this suggests that HLA-DRB1*03 alleles mediate 'resistance' to IBD. Such an effect has been proposed for insulin dependent diabetes mellitus and DRB1*15, DRB1*11.[23] The mechanisms underlying this protective effect of DRB1*03 in CD are still speculative and require further study.

The increases in DRB1*01 allele (that is, DRB1*0101, *0102 or *0103) frequencies in CD in our study is in accordance with the findings of Toyoda *et al*,[6] but with a lower p value (0·003). The p value of Toyoda *et al* (0·026) was not corrected for the number of alleles tested. However, Toyoda *et al* found a more significant association of the haplotype DRB1*01-DQB1*05 (DQB1*0501 allele usually associated with DRB1*01), than of DRB1*01 alleles alone, which suggested that DRB1 itself was not the primary locus for the association. No association of CD with DRB1*01 alleles was shown in two European studies, performed in Germany (81 patients)[8] and Sweden (118 patients).[7] The relevance of DRB1*01 as an important genetical marker thus remains open.

Smolen *et al*[5] used serological methods to show an increase in DR7 serotype in CD, but they examined very few patients (n=27) and the difference reached significance only when compared with UC. More recently, Boehm *et al*[8] used methods similar to ours and found an association between the HLA-DRB1*07 allele and CD in 81 patients from Germany. The DRB1*07 allele was also associated with psoriasis,[24] and the concurrence of psoriasis and CD in both subjects and families has been described.[25][26] Our findings might reinforce the possibility of a genetic link between the two disorders. Finally, it is perhaps significant that a viral cause for CD has been proposed[27] and DR7 has recently been shown to be associated with persistent viral infection.[28]

The differing results and the lack of a definite association between CD and HLA could be partly due to disease heterogeneity.[1][3] There is some evidence that genes in the HLA region may influence the severity of IBD. In UC, the HLA-DRB1*1502 allele has been associated with pancolitis with intractable disease[29] and with corticosteroid treatment.[30] However, in our study, using p corrected values for the number of alleles studied and for the number of different sub-groups, no significant association was observed between HLA alleles and clinical data.

In conclusion, in linkage analysis studies using the parametric lod score method[4] and, more recently, a non-parametric two point sibpair linkage method,[31] we found that HLA genes do not include the major genes for familial CD. However, this report and other association studies[6-8] suggest that HLA genes have a significant but probably minor contribution in the expression of the disease.

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