INFLAMMATORY BOWEL DISEASE

Association between K469E allele of intercellular adhesion molecule 1 gene and inflammatory bowel disease in a Japanese population


Background and aims: The genetic contribution to inflammatory bowel disease (IBD) is under investigation. Recent evidence indicates a significant linkage between a locus on chromosome 19p13 and IBD. We investigated the association between an intercellular adhesion molecule 1 gene (ICAM-1) polymorphism located on chromosome 19p13 and IBD in a Japanese population.

Methods: We compared 207 Japanese patients who had IBD (79 with Crohn’s disease [CD]; 128 with ulcerative colitis [UC]) with 103 unrelated Japanese controls. We determined R241G and K469E polymorphisms of the ICAM-1 gene using polymerase chain reaction (PCR) techniques.

Results: Both frequency and carriage rate of the K469 allele were significantly higher in IBD patients than in controls (allelic frequency, p=0.0026; carriage rate, p=0.0034; odds ratio 2.59; 95% confidence interval 1.42–4.68). Furthermore, the frequency of the K469 allele was significantly increased in both CD and UC. Subgroup analysis demonstrated that both K469 allele frequency and K469 carriage rate were significantly higher in patients with the small bowel and colon type of CD and entire colitis compared with healthy controls.

Conclusions: We identified an overall association between IBD and ICAM-1 K469 in a Japanese population. Further studies of this chromosome region are required to elucidate the gene responsible for IBD.

The aetiological relevance of ulcerative colitis (UC) and Crohn’s disease (CD) remains unknown. However, they may be associated with a genetic predisposition as familial inheritance and increased concordance rates in siblings or twins of inflammatory bowel disease (IBD) are prevalent. To understand these aetiological trends, the chromosomal region encompassing the appropriate genes requires thorough investigation.

Association studies and linkage analyses have been independently performed to identify the gene responsible for IBD. Genetic association studies have examined the relationships between HLA, DRB1, DPB1, MHC class I chain related gene A (MICA), interleukin (IL)-1α, IL-1β, and IL-1ra, tumour necrosis factor, IL-10, and intercellular adhesion molecule 1 (ICAM-1). Linkage analyses have shown a possible linkage between CD and a locus on chromosome 16 (IBD1 locus). between UC and chromosome 12q (IBD2 locus), and between IBD and chromosomes 6p (the IBD3 locus).

Any chromosomal region identified by both linkage and association analysis is likely to be closely associated with IBD and a promising source of the responsible genes. For example, microsatellite markers located on chromosome 6p, which encompasses the HLA antigen genes, indicate both a significant genetic association and linkage in IBD, and this area is being mapped to seek a candidate IBD susceptibility gene(s).

Ribou et al have identified a significant association between IBD and chromosome 19p13 using linkage analysis. Because no other linkage analyses have identified 19p13 as the region responsible for IBD, association analysis of this locus is required to confirm their findings. We therefore examined the ICAM-1 gene that is located on chromosome 19p13. ICAM-1 plays a pivotal role in the migration of neutrophils to inflammatory sites, and it may be involved in various inflammatory diseases. In addition, the ICAM-1 gene has single base polymorphisms leading to amino acid changes in codons 241 and 469, which may help to identify genetic associations in patients. Using these polymorphism markers, Yang et al could not detect an overall association between IBD (neither UC nor CD) and ICAM-1 although differences were significant when patient groups were stratified by antineutrophil cytoplasmic antibodies (ANCA).

Because few association studies of ICAM-1 and IBD have been reported, it is not known whether ICAM-1 has aetiological relevance to this disease. To precisely investigate the genetic associations of the chromosome region encompassing ICAM-1, ethnically defined populations should be analysed. A Japanese population may be suitable as the frequency of immigration is low and linkage disequilibrium decay is relatively low. In the present study, we have examined the genetic association between ICAM-1 polymorphism and IBD in a Japanese population. We found that the K469 allele frequency of the ICAM-1 gene is significantly associated overall with both CD and UC, suggesting that the 19p13 region contains potential candidates for genetic susceptibility to IBD.

MATERIALS AND METHODS

Patients and healthy controls

The study comprised 103 unrelated healthy volunteers, 128 patients with UC, and 79 with CD. All participants were Japanese and their characteristics are described in table 1. UC or CD was diagnosed by comparison with the conventional methods.

Abbreviations: UC, ulcerative colitis; CD, Crohn’s disease; IBD, inflammatory bowel disease; IL, interleukin; ICAM-1, intercellular adhesion molecule 1 gene; PCR, polymerase chain reaction; ANCA, antineutrophil cytoplasmic antibodies; OR, odds ratio.
endoscopic, histological, and clinical criteria of the Japanese Ministry of Health and Welfare with reference to the criteria provided by both the Council for International Organizations of Medical Sciences in WHO and the International Organization for the Study of Inflammatory Bowel Disease. Patients with multiple sclerosis, systemic lupus erythematosus, or other recognised autoimmune diseases were excluded from the study. All patients and controls provided written informed consent to participate in the study.

ICAM-1 genotyping

Peripheral whole blood from patients with IBD and normal controls was collected in tubes containing EDTA. Genomic DNA was extracted from samples using the Genomic PrepTM Blood DNA Isolation Kit (Amersham Pharmacia Biotech Inc., USA) according to the manufacturer's protocol. Single base polymorphisms at codon 241 (R241G) in exon 4 and at codon 469 (K469E) in exon 6 of ICAM-1 were determined using methods based on the polymerase chain reaction (PCR). The primers for amplification, PCR conditions, and allele specific oligonucleotides have been described elsewhere. The R241G polymorphism was amplified using the primers: X4L, 5′-GATTGAAGAAGCCAGCAG-3′ and X4R, 5′-GTCTGGTCCCATAGGGTAC-3′, and two end labelled allele specific oligonucleotides for the G241 allele (5′-ACAGGACACTCAGGTC-3′). The PCR products were identified by enzyme digestion with BstUI which cuts the E469 allele but not K469.

Statistical analysis

Allele and genotype frequencies between each of the patient groups and controls were compared using a 2 × 2 contingency table and the χ2 test. To correct for multiple comparisons in the subgroups, corrected p values (pC) were obtained using Bonferroni's correction factor by multiplying the p value by two (pC = 2p) because the alleles were examined simultaneously. To analyse subgroups in IBD, pC values were corrected using Fisher's exact test when sample numbers were small. After correction, pC was considered significant when <0.05. The closeness of genetic association is shown as odds ratios (OR) and 95% confidence intervals (CI).

RESULTS

Allelic frequencies, carriage rates, and genotypes of ICAM-1 alleles

Table 2 summarises allelic frequencies, carriage rates, and genotypes of the ICAM-1 gene in IBD patient groups and controls. The allelic frequency of K469 was significantly higher in patients with IBD than in controls (allelic frequency: χ2 = 10.37, p = 0.0025). This increase was also found in patients with CD (χ2 = 8.93, p = 0.0056). The carriage rate of the K469 allele in patients with CD was also higher than in controls (χ2 = 6.18, p = 0.025). Eighty nine per cent of patients with CD harboured ICAM-1 K469, giving an OR of 2.67 (95% CI 1.24–6.15) for CD. The frequency of the K469 allele was increased in UC as in CD (χ2 = 6.90, p = 0.017). The carriage rate of the K469 was also significantly higher in patients with UC than in controls (χ2 = 7.08, p = 0.016). Eighty eight per cent of patients with UC had ICAM-1 K469, giving an OR of 2.45 (95% CI 1.27–4.86).

We did not detect the R241G polymorphism in 103 individuals (50 healthy controls; 66 with UC, and 17 with CD). Because we found only the G241 allele, we did not perform an association analysis of the R241G polymorphism.

Clinical characteristics of patients with IBD harbouring the ICAM-1 K469 allele

In patients with small bowel and colon types of CD, both the allelic frequency and carriage rate of K469 allele were significantly higher than in controls (allelic frequency: pC = 0.0041; carriage rate: pC = 0.027; OR = 5.51; 95% CI 1.41–21.43) (table 3).

The allelic frequency and carriage rate of the ICAM-1 K469 allele were also significantly different in patients with the entire colitis type of UC (allelic frequency: pC = 0.045; carriage rate: pC = 0.015; OR = 2.96; 95% CI 1.33–6.53). The carriage rate

| Table 1 Characteristics of patients with Crohn’s disease and ulcerative colitis |
|-----------------------------|-----------------------------|-----------------------------|
|                            | Crohn’s disease             | Ulcerative colitis          | Healthy controls |
| n                           | 79                          | 128                         | 103             |
| Sex [M/F]                   | 42/37                       | 68/60                       | 47/56           |
| Age at onset of symptoms (y) |                             |                             |                 |
| Mean (SD)                   | 23.5 (10.1)                 | 30.8 (14.5)                 |                 |
| Range                       | 13–57                       | 11–68                       |                 |
| Affected lesion             |                             |                             |                 |
| Small and large bowel       | 31                          |                             |                 |
| Large bowel                 | 33                          |                             |                 |
| Small bowel                 | 15                          |                             |                 |
| Entire colitis              | 84                          |                             |                 |
| Left sided colitis          | 35                          |                             |                 |
| Proctitis                   | 9                           |                             |                 |
| Surgery [yes/no]            | 29/50                       | 20/108                      |                 |

Table 2 Allelic frequency and carriage rate of E/K 469 intercellular adhesion molecule 1 gene (ICAM-1) polymorphisms in patients with inflammatory bowel disease (IBD) and controls

<table>
<thead>
<tr>
<th>Genotype (n)</th>
<th>E469</th>
<th>K469</th>
<th>pC (n=103)</th>
<th>pC (n=207)</th>
<th>pC (n=79)</th>
<th>pC (n=128)</th>
<th>pC (n=128)</th>
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</thead>
<tbody>
<tr>
<td>EE</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>KK</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allelic frequency (%)</td>
<td>50.5</td>
<td>49.5</td>
<td>0.0025</td>
<td>0.0025</td>
<td>0.0056</td>
<td>0.0025</td>
<td>0.0056</td>
</tr>
<tr>
<td>Carriage rate (%)</td>
<td>74.8</td>
<td>73.8</td>
<td>0.0047</td>
<td>0.0047</td>
<td>0.0364</td>
<td>0.0037</td>
<td>0.0364</td>
</tr>
</tbody>
</table>

NS, not significant compared with healthy controls. UC, ulcerative colitis; CD, Crohn’s disease.
of K469 in left sided UC was also significant as well as in the entire colitis type of UC (left sided type: 88.6%; entire type: 89.3%). However, these findings were not significant compared with the proctitis type of UC and healthy controls (proctitis type: 66%; controls: 73.8%) (table 4).

**DISCUSSION**

Considerable effort has been made to identify IBD susceptibility genes (either CD or UC). Most of these efforts were attempted using genetic association studies and linkage analyses. However, because IBD is highly multifactorial in terms of both environmental and genetic factors, precise mapping of the susceptible loci should be complex. Coordinate examinations using both of the methods mentioned above are needed to search for the potent IBD loci. The present study attempted using genetic association studies and linkage analyses. Linkage disequilibria between genes in the same chromosomal region may vary among different ethnic populations.

As the Japanese are thought to have expanded from a founder population of approximately 1000 individuals over 100 generations, they are considered suitable for both genetic association and linkage analyses. Linkage disequilibria between ICAM-1 and the responsible loci for IBD may have been highly conserved on chromosome 19p13 within this population but more detailed linkage analyses of this chromosome region are required to identify the IBD susceptibility gene(s) in this population.

Several investigators have examined the genetic association between ICAM-1 gene polymorphism and chronic inflammatory diseases. Among Polish Caucasian patients with multiple sclerosis, the allelic frequency of ICAM-1 K469 was significantly increased (68% vs 49% in controls) while the distribution of the R241G polymorphism was not significant. In Italian patients with either polymyalgia rheumatica or giant cell arteritis, the frequency of the R241 allele was significantly higher than in controls (polymyalgia rheumatica/giant cell arteritis 12.9% vs 3.1% in controls) but the K469E polymorphism was not significant. The allelic frequency of E469 was significantly increased among Palestinian and Jordanian patients with Behçet's disease (47.6% vs 38.3% in controls) but the R241G polymorphism was not identified. These results are contradictory and so the significance of this gene may differ according to the type of inflammatory disease.

All of these diseases as well as IBD are characterised by intense infiltration of leucocytes at inflamed lesions. ICAM-1 is expressed on the surface of activated endothelial cells where diabetes. The ICAM-1 polymorphism was significantly associated with adult onset diabetes in a Japanese population but not in Danish or Finnish families. Linkage disequilibria between genes in the same chromosomal region may vary among different ethnic populations.

**Table 3** Allelic frequencies and carriage rate of intercellular adhesion molecule 1 (ICAM-1) alleles in patients with Crohn's disease by affected lesions

<table>
<thead>
<tr>
<th>Genotype (n)</th>
<th>Carriage rate (%)</th>
<th>Allelic frequency (%)</th>
<th>p&lt;sub&gt;i&lt;/sub&gt;</th>
<th>Carriage rate (%)</th>
<th>Allelic frequency (%)</th>
<th>p&lt;sub&gt;i&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>E69</td>
<td>50.5</td>
<td>28.8</td>
<td>0.00411</td>
<td>35.5</td>
<td>NS</td>
<td>46.6</td>
</tr>
<tr>
<td>K69</td>
<td>49.5</td>
<td>71.2</td>
<td>0.00411</td>
<td>64.5</td>
<td>NS</td>
<td>53.3</td>
</tr>
</tbody>
</table>

**Table 4** Allelic frequencies and carriage rate of intercellular adhesion molecule 1 (ICAM-1) alleles in patients with ulcerative colitis by affected lesions

<table>
<thead>
<tr>
<th>Genotype (n)</th>
<th>Carriage rate (%)</th>
<th>Allelic frequency (%)</th>
<th>p&lt;sub&gt;i&lt;/sub&gt;</th>
<th>Carriage rate (%)</th>
<th>Allelic frequency (%)</th>
<th>p&lt;sub&gt;i&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>E69</td>
<td>50.5</td>
<td>38.7</td>
<td>0.0452</td>
<td>34.3</td>
<td>0.0377</td>
<td>50.0</td>
</tr>
<tr>
<td>K69</td>
<td>49.5</td>
<td>61.3</td>
<td>0.0452</td>
<td>65.7</td>
<td>0.0377</td>
<td>50.0</td>
</tr>
</tbody>
</table>

NS, not significant compared with healthy controls.
it plays an important role in lymphocyte migration and activation. R241G and K469E amino acid changes in the ICAM-1 gene may influence the functional role of ICAM-1 as both are located on the Mac-1 binding domain and in immunoglobulin-like domain 5, respectively. The relevance of the ICAM-1 gene polymorphism may be explained by functional alteration of this gene product.23,25

Recent linkage analyses have revealed that the chromosome region 19p13 is closely related to the atopy phenotype in Italian families with allergic asthma and type 1 diabetes in a British population.20 This chromosomal region encompasses several important genes that regulate the immunological and inflammatory systems (complement component 3, thromboxane A2 receptor, leukotriene B4 hydroxylase, Janus protein tyrosine kinase family).20 Our results should be confirmed when the genetic association of these loci is determined. In conclusion, the present study revealed an overall association between the ICAM-1 K469 allele and IBD (CD and UC) and may potentiate the significance of chromosome 19p13. Further studies are required to confirm our findings.

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