Pepsinogen C gene polymorphisms associated with gastric body ulcer

T Azuma, N Teramae, T Hayakumo, K Yasuda, M Nakajima, T Kodama, H Inokuchi, K Hayashi, R T Taggart, K Kawai

Abstract
This study was aimed to investigate the association of restriction fragment length polymorphisms (RFLPs) for pepsinogen genes with peptic ulcer disease. Eighty unrelated controls, 61 patients with gastric ulcer, and 57 patients with duodenal ulcer were studied. No genetic polymorphisms for pepsinogen A were detected by EcoRI digestion in Japanese subjects but a 100 base pairs insertion-deletion RFLP for the pepsinogen C gene was observed. The allele frequencies of the large (3-6 kilobase EcoRI fragment) and the small fragment (3-5 kilobase EcoRI fragment) were 80-6% and 19-4% respectively in controls, 55-4% and 44-6% in patients with gastric body ulcer, 79-4% and 20-6% in patients with gastric ulcer, 71-4% and 28-6% in patients with gastric antral ulcer, and 75-4% and 24-6% in patients with duodenal ulcer. The allele frequency of the small fragment was significantly higher in patients with gastric body ulcer than in controls and in patients with gastric angular or antral ulcer. The genotypes which possessed the small fragment were significantly more frequent in patients with gastric body ulcer (78-4%) than in controls (33-8%) and in patients with gastric angular or antral ulcer (37-5%). These results suggest that there is a significant association between the genetic polymorphism at the pepsinogen C gene locus and gastric body ulcer, and that the pepsinogen C RFLP is a useful marker of the genetic predisposition to this disorder. These results also indicate genetic heterogeneity of gastric ulcer disease, and suggest that the pepsinogen C RFLP may be a useful subclinical marker to explain the differences in genetic aetiology of gastric body ulcer and gastric angular or antral ulcer.

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The basis for supposing that there are hereditary factors in the aetiology of peptic ulcer disease is provided by studies showing aggregation of peptic ulcers in the families of affected individuals to a greater extent than that found in the general population. More important information about the influence of hereditary factors in ulcer disease has been derived from studies of twins. It has been reported that the concordance rate for peptic ulcer in monozygotic twins is greater than in dizygotic twins. Since the concordance was less than 100%, the values could also be interpreted as indicating interactions between genetic predisposition and environmental factors. A number of inherited characteristics, such as blood groups and HLA antigens, have been studied to determine the degree of association with peptic ulceration. In this connection, it is assumed that a sufficiently positive association between peptic ulceration and the allele of a defined genetic locus indicates that the genetic trait plays a part in the pathogenesis of the ulceration.

Efforts to understand the genetics of common chronic diseases such as hypertension, diabetes mellitus, and hypercholesterolaemia have focussed on the identification of DNA markers. Restriction fragment length polymorphisms (RFLPs) can be very powerful tools in these studies. RFLPs can act as markers of differences between individuals at the gene level. Analysing RFLPs may identify alleles at a particular genetic locus that are associated with a clinical phenotype.

Human pepsinogen, the inactive precursor of pepsin, comprises two biochemically and immunologically distinct groups of isozymogens: namely, pepsinogen A (PGA) (previous nomenclature: PGI) and pepsinogen C (PGC) (previous nomenclature: PGII). PGA is the precursor of pepsin A. PGC, also known as progastricin, is the precursor of pepsin C or gastricin. These two types ofzymogens differ in their topological distribution within the stomach. PGA is localised mainly in the fundus, whereas PGC is distributed throughout the stomach and proximal duodenum. PGC consists of five electrophoretic isozymogens (Pg 1–5) and is remarkably heterogeneous, as evidenced by an extensive protein electrophoretic polymorphism resulting from multiple haplotypes containing different combinations of the individual PGA genes and one or more post-translational modifications of the primary gene products. Two principal forms of the PGA polymorphisms are recognised: phenotype A, which possesses all five PGA isozymogens; and phenotype B, which lacks Pg 5. In contrast, PGC consists of two electrophoretic isozymogens (Pg 6 and Pg 7). No genetic variation has been described at the protein level but at the DNA level an insertion-deletion RFLP is observed with several restriction enzymes.

It has been reported that there is an association between duodenal ulcer and PGA phenotype A. In addition, two families with a prominent history of duodenal ulcer have been described in whom a high level of serum PGA (hyperpepsinogenaeina I) is inherited as an autosomal dominant trait with a high risk of developing duodenal ulcer. From these findings, it can be suggested that pepsinogen genes are involved in the pathogenesis of peptic ulcer. The aim of the present study was to analyse RFLPs for PGA and PGC, and to examine an association between peptic ulcer and the RFLPs.
Patients and methods

PATIENTS

One hundred and eighteen patients were studied - 61 with gastric ulcer (41 men and 20 women, age range 19–82 years, mean 56.5 years) and 57 with duodenal ulcer (35 men and 22 women, age range 18–85 years, mean 45.8 years). Most of the patients were referred for investigation to the Department of Gastroenterology of Kyoto Second Red Cross Hospital, and the remainder were seen at the Third Department of Internal Medicine, Kyoto Prefectural University of Medicine. Peptic ulcer was diagnosed endoscopically. No patient had a history of taking ulcerogenic drugs, such as aspirin, indomethacin, and steroids. All patients had a history of ulcer recurrence confirmed by endoscopy. Patients with combined duodenal and prepyloric ulcer were excluded. The 80 unrelated control subjects with no evidence of peptic ulcer disease (40 men and 40 women, age range 30–81 years, mean 53.3 years) were taken from subjects who visited multiphasic health testing services held by Department of Preventive Medicine, Kyoto Prefectural University of Medicine. The services included endoscopic screening for gastric cancer, which is a leading cause of cancer deaths in Japan. No control subject had evidence of peptic ulcer by endoscopy. All patients and controls were living in Kyoto Prefecture and were Japanese in origin. The study protocols were approved by each institution's human subjects' protection committee.

SOUTHERN ANALYSIS

Genomic DNA was purified from whole blood, digested with EcoRI (Toyobo, Tokyo, Japan) according to the manufacturer's instructions, and subjected to electrophoretic separation in agarose gels. Genomic DNA was denatured and transferred to nitrocellulose, and prehybridised at 42°C for 3 hours in 50% formamide, 25 mM sodium phosphate (pH 6.5), 500 µg/ml sonicated salmon testes DNA, and 5× Denhardt’s. Filters were hybridised overnight in a solution containing 50% formamide, 10% dextran sulphate, 20 mM sodium phosphate (pH 6.5), 250 µg/ml sonicated salmon testes DNA, 2× Denhardt’s, and PGA or PGC cDNA probes (1×10⁶ cpm/ml) labelled with [32P]-dCTP by the random primer method as described previously. The PGA cDNA probe (pPGA101) is a full length cDNA including exons 1–9 of the predicted human PGA coding sequence and portion of the 5' and 3' untranslated region. The PGC cDNA probe (PGC301) is a partial cDNA including exons 2–9 of the predicted human PGC coding sequence and portion of the 3' untranslated region. Filters were rinsed several times at room temperature in a solution containing 0.1× SSC and 0.05% sodium dodecyl sulphate (SDS) to remove excess hybridisation solution and then washed at 55°C for 15 minutes in the same solution for detection of the PGA or PGC sequences.

SERUM PGA AND PGC VALUES

Serum was obtained after an overnight fast, and was stored at −20°C until analysed. Serum PGA and PGC values were determined by PGA and PGC RIA BEAD kits (Dainabot Co, Tokyo, Japan). The limits of the detection were 0.1 ng/ml for PGA and 0.63 ng/ml for PGC. The intra-assay coefficients of variation were 5.0% (n=5) for PGA and 3.5% (n=5) for PGC. The inter-assay coefficients of variation were 8.0% (n=5) for PGA and 6.5% (n=5) for PGC.

STATISTICAL ANALYSIS

The significance of the association between peptic ulcer and pepsinogen RFLPs was tested using the χ² test, with significance assigned values better than p<0.05. The logit estimate of the common odds ratio was calculated using the method of Woolf. The significance of the differences in serum PGA and PGC values and the ratio of PGA to PGC was determined by the Student’s t test for unpaired samples, with significance assigned values better than p<0.05.

Results

RFLP FOR PGA

Four EcoRI restriction fragments (17.8, 17.2, 13.5, and 3.9 kilobase (kb)) were observed by Southern analysis for PGA. We did not detect any RFLPs for PGA in Japanese populations (Fig 1).

RFLP FOR PGC

Four EcoRI restriction fragments (20, 5.7, 3.6, and 3.5 kb) were observed among the genotypes, of which two were polymorphic (3.6 and 3.5 kb) by Southern analysis for PGC (Fig 2). The polymorphic pattern was identical to that reported in white populations. Of the 80 unrelated control subjects, 53 were homozygous

Figure 1: Southern blot analysis of genomic DNA from seven unrelated individuals digested with EcoRI. The nitrocellulose blot was hybridised with a PGA cDNA probe (PGA101). RFLPs for PGA were not observed in Japanese populations. The sizes of hybridising to the probe are indicated in kilobases.
for the 3.6 kb fragment (3-6/3-6), 23 were heterozygous – that is, they possessed both the 3.6 kb and the 3.5 kb fragment; 3-6/3-5 kb – and four were homozygous for the 3.5 kb fragment (3-5/3-5) (Table I). Hence, the estimated allele frequencies for the large and the small fragments were 80-6% and 19-4%, respectively, and were not significantly different from those in white populations (82.5% and 17.5% respectively).23

PGC RFLP IN PATIENTS WITH GASTRIC ULCER
Of 61 patients with gastric ulcer, 23 were homozygous for the large fragment (3-6/3-6), 32 were heterozygous for the RFLP (3-6/3-5), and six were homozygous for the small fragment (3-5/3-5). The allele frequencies of the large and the small fragments were 63-9% and 36-1%, respectively (Table I). The increased frequency of the small fragment in the patients with gastric ulcer (36.1%) compared with controls (19.4%), was statistically significant ($\chi^2 = 23.83; p < 0.005$), and it yielded an odds ratio of 2-35 (95% confidence limits, 1-37 to 4-02) (Table I).

PGC RFLP IN PATIENTS WITH DUODENAL ULCER
Of 57 patients with duodenal ulcer, 30 were 3-6/3-6, 26 were 3-6/3-5, and one was 3-5/3-5 (Table I). The allele frequencies of the large and the small fragments were 75.4% and 24.6% respectively in patients with duodenal ulcer, and were not significantly different from those in controls.

PGC RFLP ANALYSIS ACCORDING TO SEX
In no group were there significant differences between men and women in respect of allele frequencies. In both sexes, the increased frequency of the small fragment in patients with gastric ulcer (35.4% in men, 37.5% in women) compared with controls (18.8% in men, 20.0% in women), was statistically significant (Table I).

PGC RFLP ANALYSIS ACCORDING TO THE LOCATION OF GASTRIC ULCER
The allele frequency of the small fragment in patients with gastric ulcer was analysed according to the location of the gastric ulcer. The frequency of the small fragment was 44.6% in patients with gastric body ulcer, 20.6% in patients with gastric angular ulcer, and 28.6% in patients with a gastric antral ulcer. The increased frequency of the small fragment in patients with gastric body ulcer (44.6%), compared with controls (19-4%) was statistically significant ($\chi^2 = 16.20; p < 0.005$), and it yielded an odds ratio of 3-34 (95% confidence limits, 1-83 to 6-12) (Table II). In order to gain a better appreciation of the genotypic risk, the relative risk of gastric body ulcer associated with the genotypes was estimated. The relative risk of gastric body ulcer associated with the presence of the small fragment (3-6/3-5 and 3-5/3-5) compared with 3-6/3-6 was 7.12 (95% confidence limits, 2.87 to 17.67) and was statistically significant ($\chi^2 = 20.2; p < 0.005$). The relative risk associated with 3-5/3-5 compared with 3-6/3-6 was 6.63 (95% confidence limits, 1.37 to 31.93) and was statistically significant ($\chi^2 = 6.7; p < 0.01$). The relative risk associated with 3-6/3-5 compared with 3-6/3-6 was 7.20 (95% confidence limits, 2.83 to 18.33) and was statistically significant ($\chi^2 = 19.32; p < 0.005$).

SERUM PGA AND PGC VALUES AND THE RATIO OF PGA TO PGC
Serum PGA values were significantly higher in patients with duodenal ulcer than in controls and in patients with gastric ulcer. Serum PGA values were significantly higher in patients with gastric ulcer and in patients with duodenal ulcer than in controls. The PGA/PGC ratio was significantly lower in patients with gastric ulcer than in controls and in patients with duodenal ulcer (Table III).

Serum PGA and PGC values and the PGA/PGC ratio were analysed according to the
TABLE II  Distribution of the pepsinogen C restriction fragment length pattern according to the location of gastric ulcer

<table>
<thead>
<tr>
<th>Location of gastric ulcer</th>
<th>Restriction fragment pattern</th>
<th>Allele frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3'-6/3-6</td>
<td>3'-6/3-5</td>
</tr>
<tr>
<td>Body (n=37):</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>Upper body</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Middle body</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Lower body</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>8</td>
<td>8</td>
</tr>
<tr>
<td>Antrum (n=7)</td>
<td>11</td>
<td>5</td>
</tr>
<tr>
<td>Control (n=50)</td>
<td>33</td>
<td>15</td>
</tr>
</tbody>
</table>

*Significantly different from controls, $\chi^2$=9.6; p=0.005.
**Significantly different from controls, $\chi^2$=6.2; p=0.01.
### TABLE III  Serum pepsinogen A (PGA) and pepsinogen C (PGC) values (ng/ml) and the ratio of PGA:PGC [mean (SEM)]

<table>
<thead>
<tr>
<th></th>
<th>PGA</th>
<th>PGC</th>
<th>PGA/PGC ratio</th>
<th>Age (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric ulcer:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body (n=37)</td>
<td>44-10 (4-49)</td>
<td>18-02 (1.41)</td>
<td>2-49 (0.13)</td>
<td>57-7 (2.1)</td>
</tr>
<tr>
<td>Angle (n=50)</td>
<td>57-17 (5-05)</td>
<td>18-73 (1.84)</td>
<td>5-64 (0.35)</td>
<td>54-7 (2.8)</td>
</tr>
<tr>
<td>Total (n=61)</td>
<td>48-63 (5-42)</td>
<td>18-48 (1.45)</td>
<td>5-02 (0.18)</td>
<td>56-5 (1.8)</td>
</tr>
<tr>
<td>Duodenal ulcer (n=57)</td>
<td>65-41 (3-02)</td>
<td>17-78 (1.00)</td>
<td>3-91 (0.14)</td>
<td>45-8 (2.0)</td>
</tr>
<tr>
<td>Controls (n=80)</td>
<td>43-31 (2-68)</td>
<td>14-05 (1.02)</td>
<td>3-00 (0.25)</td>
<td>53-3 (1.6)</td>
</tr>
</tbody>
</table>

a: significantly different from controls (p=0.05). b: significantly different from controls (p=0.05) and patients with antral ulcer (p=0.005). c: significantly different from controls (p=0.005). d: significantly different from controls (p=0.05). e: significantly different from controls (p=0.05). f: significantly different from controls (p=0.005) and patients with duodenal ulcer (p=0.005). g: significantly different from controls (p=0.005) and patients with gastric ulcer (p=0.005). h: significantly different from controls (p=0.005). i: significantly different from controls (p=0.05) and patients with gastric ulcer (p=0.005).

Discussion

A major difficulty in genetic studies of peptic ulcer disease has been the lack of subclinical markers. It has been reported that blood group O and non-secretor status are associated with duodenal ulcer,1 however, the magnitude of the association was very weak. The relative risk of developing a duodenal ulcer for persons with blood group O and non-secretor status was 1.6-5 and 1.5 respectively.2 Studies of the prevalence of HLA antigens in patients with duodenal ulcer have shown significant increases in the frequency of the antigens HLA-B5, HLA-B12, and HLA-BW35.3,4 However, the contribution of these HLA antigens to ulcer prevalence is small because of their low prevalences in populations, even if the magnitude of the associations is relatively high.

Our data indicate an association between the genetic polymorphism at the PGA gene locus and gastric body ulcer. The allele frequency of the small fragment of the PGC RFLP in patients with gastric body ulcer (44.6%) was significantly higher than that in controls (19.4%). The odds ratio of gastric body ulcer associated with the presence of the lower fragment of the PGC RFLP was 3.44, and was statistically significant. The genotypes containing the small fragment (3'-6/3-5 and 3'-5/3-5) were significantly more frequent in patients with gastric body ulcer (78.4%) than in controls (33.8%) and in patients with gastric angular or antral ulcer (37.5%). The relative risk of genetic body ulcer associated with the presence of the small fragment as compared with the homozygosity for the large fragment was 7.12. This value was greater than those of the associations between duodenal ulcer and blood group O (1.35), non-secretor status (1.5), and HLA B5 (2.9).5,6 These results suggest that the PGC RFLP is a useful subclinical marker of the genetic predisposition to gastric body ulcer.

In addition, our data indicate genetic heterogeneity of gastric ulcer disease. The allele frequency of the small fragment of the PGC RFLP was significantly higher in patients with gastric body ulcer than in patients with gastric angular or antral ulcer. The concept of genetic heterogeneity implies that a particular clinical disorder is a group of distinct diseases with different aetiologies, both genetic and non-genetic. Some degree of heterogeneity has already been shown in peptic ulcer disease. Doll and Kellock showed the independent segregation of gastric and duodenal ulcers. They also concluded that genetic factors alone, without environmental interactions, could account for this segregation.6 Other subgroups have been defined by identifying pathophysiological disturbances, such as gastric hypersecretion, increased rate of gastric emptying, or increased gastrin release, and combining these abnormalities with genetic markers.3,6 These findings speculate that there are the differences in the genetic aetiology between gastric body ulcer and gastric angular or antral ulcer, and suggest that the PGC RFLP may be a useful subclinical marker to separate the genetic differences.

The meaning of the association is not clear in this study, however, several hypotheses can be proposed. One is that the PGC gene itself is one of the genes responsible for gastric body ulcer. Previous studies of the PGC genomic clone suggested that the RFLP involves a 100 bp insertion or deletion of intron sequence located between exons 7 and 8.24 It might be possible that this rearrangement of the gene reflects differences in gastric mucosal structure and function between patients with gastric body ulcer and patients with gastric angular or antral ulcer and patients with duodenal ulcer. To analyse the meaning of the association, serum PGA and PGC values and the PGA/PGC ratio were examined in the present study. There were no significant differences in serum PGA and PGC values and in the PGA/PGC ratio among the genotypes of the PGC RFLP but serum PGA and PGC values were significantly higher in patients with duodenal ulcer and in patients with gastric angular or antral ulcer than in controls. In contrast, serum PGC values were significantly higher and the PGA/PGC ratio was significantly lower in patients with gastric body ulcer than in controls, in patients with duodenal ulcer, and in patients with gastric angular or antral ulcer. These results agree with previous reports.7,8 Samloff et al reported that a raised pepsinogen I (PGA) con-
centration is a major risk factor for duodenal ulcer, whereas a high serum pepsinogen II (PGC) concentration and a low PGA/PGC ratio are major risk factors for gastric ulcer. These differences in serum PGA and PGC values and in the PGA/PGC ratio seem to reflect differences in gastric mucosal structure and function between patients with gastric body ulcer and patients with duodenal ulcer and patients with gastric angular or antral ulcer. One difference is that the fundic gland mucosa is often normal in patients with duodenal ulcer, but is affected by atrophic gastritis in those with gastric ulcer, especially high lying gastric ulcer. It has been reported that the combination of high PGC value and a low PGA/PGC ratio is a subclinical marker of atrophic gastritis. Tatsuta and Okuda examined the influence of fundal gastritis on the location of gastric ulcers by the endoscopic Congo red test. Their results indicated that ulcers were more proximal when atrophic gastritis was more severe. These findings suggest that the rearrangement of the PGC gene may reflect the atrophic change of gastric mucosa. Atrophic gastritis is thought to be the precursor lesion of the intestinal type of gastric carcinoma which is the most common cancer in Japan. Further studies are now underway to examine the association between gastric cancer and the PGC-RFLP. Helicobacter pylori has recently been implicated as an important aetiological factor of gastritis and gastroduodenal ulceration. Further analysis of the relationship between the PGC RFLP and the H pylori status and precise histological changes are clearly needed in the future studies.

Another possible explanation of the association between the PGC RFLP and gastric ulcer disease is that the PGC gene is not itself responsible for the predisposition but one of the responsible genes is closely linked to it. It is interesting to note that the PGC gene was localised to human chromosome 6p21.1-pter by analysis of mouse x human somatic cell hybrids. Our recent linkage analysis showed that the PGC gene is closely linked to the HLA cluster which has been investigated to determine the association with peptic ulcer disease. Further molecular biological studies using RFLP markers in this chromosome region will clarify this. Lastly, the presence of reduced penetrance, other modifier genes, or an interaction with the environment may explain the association.

Human PGA has been known to be a complicated protein polymorphism. Recent molecular studies with cDNA probes have provided evidence that PGA haplotypes containing different combinations of genes are the major contributing factor in the observed protein heterogeneity. The PGA gene complex located at 11q13 includes several different haplotypes that contain variable numbers of PGA genes each of which encodes an electrophoretically distinguishable isozymogen; Pg5 (PGA5), Pg4 (PGA4), and Pg3 (PGA3). As a result of the multiple gene variation, more than 50% of white people are heterozygous. Five EcoRI restriction fragments are detected among phenotypes (22, 17-8, 17-2, 13-5, and 3-9 kb). The 13-5 kb fragment is present in all phenotypes that contain the A haplotype and is absent in the B haplotype. Contrary, four EcoRI restriction fragments (17-8, 17-2, 13-5, and 3-9 kb) were observed in the present study, and we did not detect any RFLPs for PGA in Japanese populations by EcoRI digestion. These findings indicate that all Japanese are homozgyous for the A haplotype for PGA. Samloff et al also reported that all persons of Far Eastern ancestry were phenotype A of PGA by the analysis of the protein heterogeneity. Several genetic and environmental influences have been proposed to explain the racial and the geographic variability in the incidence of peptic ulcer disease. It is probable that many factors are involved in the incidence, and that these vary among populations. It has been reported that PGA phenotype A is associated with duodenal ulcer in white people. The relative risk of the association with PGA phenotype A was 1.74. If the PGA genes are on the average linked (in disequilibrium) to one of responsible genes for duodenal ulcer, the association is expected to vary among populations.

RFLP for PGA has been identified both in white people and in Japanese. The frequencies of the RFLP alleles were near identical between the two groups. The racial difference of the association between genetic polymorphisms of PGA and gastric body ulcer remains unclear in this study. The incidence of gastric ulcer is higher than that of duodenal ulcer in Japan. In our previous study, the ratio of gastric to duodenal ulcers was 1.69 in Kinki district where Kyoto Prefecture is located. In contrast, duodenal ulcer is more common than gastric ulcer in most western countries. Bonnevie reported that the incidence rate of duodenal ulcer was four times higher than that of gastric ulcer in Copenhagen County. Kurata et al reported that duodenal ulcers were diagnosed 2-5 times more frequently than gastric ulcers in Los Angeles, California. The similar distribution of the RFLP alleles in Japanese and whites could not explain the difference of the ratio of gastric to duodenal ulcers in these populations. Studies are now underway to confirm our results in a larger number of patients and in different populations. Peptic ulcer disease is a multifactorial inherited disease. There are interactions between genetic predisposition and environmental factors. In addition, peptic ulcer disease represents a heterogeneous group of disorders attributable to a variety of genetic and environmental causes. With the larger numbers that can be attained, attempts will be made to correlate our findings with various clinical features and environmental factors, to analyse the meaning of the association we described in the present study.

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19 Taggart RT, Mohandas TK, Shows TB, Bell GI. A gene complex containing variable numbers of pepsinogen genes is located in the centromeric region of the human chromosome 11 and determines the high frequency electrophoretic polymorphism. Proc Natl Acad Sci USA 1985; 82: 6240-4.


