Association between genetic polymorphism of the pepsinogen C gene and gastric body ulcer: the genetic predisposition is not associated with *Helicobacter pylori* infection

Y Ohtaki, T Azuma, J Konishi, S Ito, M Kuriyama

Abstract

***Background and aims*—The genetic trait plays a part in the pathogenesis of peptic ulcer disease. To identify a DNA marker for peptic ulcer disease, the association between the restriction fragment length polymorphism (RFLP) of the pepsinogen C (PGC) gene and peptic ulcer disease was investigated.**

***Patients and methods*—One hundred and seventy seven unrelated controls, 75 patients with gastric ulcer, and 70 with duodenal ulcer were studied. PGC-RFLP was analysed by polymerase chain reaction (PCR), and the association between PGC-RFLP and peptic ulcer disease was examined. The relation between the genetic association of PGC polymorphism with peptic ulcer and *Helicobacter pylori* infection was also examined.**

***Results*—Four alleles, 480 (allele 1), 450 (allele 2), 400 (allele 3), and 310 bp (allele 4), were detected by PCR. The frequency of allele 4 was significantly higher in patients with gastric body ulcer than in controls (χ^2=9.92, p<0.005). Genotypes containing allele 4 were significantly more frequent in patients with gastric body ulcer than in controls and patients with gastric angular or antral ulcer. The relative risk of gastric body ulcer associated with the presence of allele 4, compared with its absence, was 4.63 and was statistically significant (χ^2=14.84, p<0.005). There were no significant differences in the allelic frequencies between *H pylori* positive and *H pylori* negative groups in controls, patients with gastric body ulcer, or patients with gastric angular or antral ulcer. Both in *H pylori* negative and *H pylori* positive cases, there was an increased frequency of allele 4 in patients with gastric body ulcer compared with controls.**

***Conclusions*—These results suggest that there is a significant association between this genetic polymorphism at the PGC gene locus and gastric body ulcer. There are differences in the genetic aetiology between gastric body ulcer and gastric angular or antral ulcer. PGC-RFLP may be used as a genetic marker for a genetic predisposition to gastric body ulcer; this genetic predisposition is not associated with *H pylori* infection.**

(*Gut* 1997; **41:** 469–474)

Keywords: pepsinogen C; gastric ulcer; genetic heterogeneity; restriction fragment length polymorphism; *Helicobacter pylori*

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Accepted for publication
25 March 1997

Family and twin studies have demonstrated that peptic ulcer disease is inherited as a multifactorial trait, and that several different genes may be involved in its pathogenesis.[1–5] Efforts to understand the genetics of common chronic diseases such as hypertension, diabetes mellitus, and hypercholesterolaemia have focused on the identification of DNA markers.[6–8] The use of restriction fragment length polymorphisms (RFLPs) can be very powerful. RFLPs can act as markers of differences between individuals at the gene level. Analysis of RFLPs for specific candidate genes may identify alleles at a particular locus that are associated with a clinical phenotype. The most popular current hypothesis proposes that ulcers are caused by, and persist, due to the breakdown of a supposed equilibrium between aggressive and defensive factors. Pepsin is one of the major aggressive factors in peptic ulcer disease. In this study, we selected pepsinogen genes as candidate genes in an effort to identify DNA markers for peptic ulcer disease.

Human pepsinogen is the inactive precursor of pepsin, and comprises two biochemically and immunologically distinct groups of isozymogens: PGA (previously known as PGI) and PGC (previously known as PGII).[9–12] PGA is the precursor of pepsin A. PGC, also known as progastricsin, is the precursor of pepsin C or gastricsin. PGA has five electrophoretic isozymogens (Pg 1–5) and is remarkably heterogeneous as shown by an extensive protein electrophoretic polymorphism resulting in multiple haplotypes containing different combinations of the individual PGA genes and one or more post-translational modifications of the primary gene products.[13 14] Two principal forms of PGA polymorphism have been recognised: phenotype A, which possesses all five PGA isozymogens; and phenotype B, which lacks Pg 5.[15] In our previous study, however, no RFLPs for PGA were detected in the Japanese populations. We concluded that all Japanese are homozygous for the A haplotype of PGA.[16] In contrast, PGC has two electrophoretic

isozymogens (Pg 6 and Pg 7).[9 10] No genetic variation has been described at the protein level. However at the DNA level, a 100 bp insertion-deletion RFLP located between exons 7 and 8 was observed with several restriction enzymes.[17 18] The RFLP for PGC was identified in both Caucasians and Japanese. We have shown that there is a significant association between genetic polymorphisms at the PGC gene locus and gastric ulcer disease, as determined by Southern blotting, and that there is genetic heterogeneity in this disease; this depends on the location of the gastric ulcer.[16] We developed a polymerase chain reaction (PCR) system to identify the RFLP for PGC. PCR is highly efficient at detecting DNA polymorphisms. Alleles which cannot be resolved by Southern blotting are easily resolved on conventional agarose gels. PCR permitted three alleles to be discriminated, all of which had previously been classified as the upper allele of the insertion-deletion PGC-RFLP by Southern blotting.[19]

In this study we analysed the PGC-RFLP of patients with peptic ulcer by PCR, and examined the association between PGC-RFLP and the gastric body ulcer. *Helicobacter pylori* infection is widely accepted to be the predominant cause of chronic gastritis, which is strongly associated with gastric ulcer, duodenal ulcer, and gastric cancer. The pathogenic mechanisms whereby *H pylori* causes human diseases remain unknown. We therefore also examined the relation between the genetic association of the PGC polymorphism with gastric body ulcer and *H pylori* infection.

Patients and Methods

PATIENTS

A total of 145 patients, 75 with gastric ulcer (45 men and 30 women, age range 19–82 years, mean 56.3) and 70 with duodenal ulcer (44 men and 26 women, age range 18–85 years, mean 45.4) was studied. All patients were referred for investigation to the Second Department of Internal Medicine, Fukui Medical School. Peptic ulcer was diagnosed endoscopically. None of the patients had a history of taking ulcerogenic drugs, such as aspirin, indomethacin, or steroids. All had a history of recurrent ulceration, diagnosed by endoscopy. Patients with combined duodenal and prepyloric ulcers were excluded from this study. A total of 177 unrelated controls with no evidence of peptic ulcer disease (100 men and 77 women, age range 30–81 years, mean 52.8) were selected from individuals undergoing multiphasic health testing (including endoscopic examination to screen for gastric cancer) at the Second Department of Internal Medicine, Fukui Medical School. All controls had no evidence of peptic ulcer by endoscopy. All patients and healthy controls were living in Fukui Prefecture and were Japanese. This study was performed according to the principles of the Declaration of Helsinki, and consent was obtained from each patient after full explanation of the study.

SOUTHERN BLOTTING

Genomic DNA was purified from whole blood, digested with EcoRI (Toyobo, Tokyo, Japan) following the manufacturer's instructions, and separated by electrophoresis in an agarose gel. Genomic DNA was denatured and transferred to nitrocellulose[20] and prehybridised at 42°C for three hours in 50% formamide, 25 mM sodium phosphate (pH 6.5), 500 μg/ml sonicated salmon testes DNA, and 5× Denhardt's solution. Membranes were hybridised overnight in a mixture containing 50% formamide, 10% dextran sulphate, 20 mM sodium phosphate (pH 6.5), 250 μg/ml sonicated salmon testes DNA, 2× Denhardt's solution, and PGC cDNA probe (1×10^6 cpm/ml) labelled with ^{32}P-dCTP by the random primer method as described previously.[21] The PGC cDNA probe (PGC301) is a partial cDNA including exons 2–9 of the predicted human PGC coding sequence and a portion of the 3' untranslated region.[22] The membranes were rinsed several times at room temperature in 0.1× SSC containing 0.05% sodium dodecyl sulphate (SDS), then washed at 55°C for 15 minutes in the same solution to detect the PGC sequences.

PCR ANALYSIS

Genomic DNA (1 μg) was amplified with 2.5 units of DNA polymerase from *Thermus aquaticus* as described before. The oligonucleotide primers chosen were: upstream, 5'-AGC CCTAAGCCTGTTTTTGG-3'; and downstream, 5'-GGCCAGATCTGCGTGTTTTA -3'.[19] Buffers for PCR amplification have been described previously.[19] The reaction mixture, including 25 pmol of each primer, was subjected to 30 cycles of one minute at 94°C, one minute at 63°C, and two minutes at 72°C; with a final extension at 72°C for seven minutes. The amplification reactions proceeded in a PerkinElmer Cetus Thermocycler (model PCR 400). One tenth of the reaction mixture (50 μl total volume) underwent electrophoresis in a 3% agarose gel, and the gel was stained with ethidium bromide.

SERUM PGA AND PGC CONCENTRATIONS

Serum was obtained after an overnight fast, and stored at –20°C until analysed. Serum PGA and PGC concentrations were determined by PGA and PGC RIABEAD kits (Dainabot Co., Tokyo, Japan).[23] The limits of detection were 0.1 and 0.63 ng/ml for PGA and PGC, respectively. 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.

DIAGNOSIS OF *H PYLORI* INFECTION

The distribution of *H pylori* is uneven in the gastric mucosa. Although there has been considerable improvement in the techniques used for the diagnosis of *H pylori* infection, several studies have now conclusively demonstrated that a combination of at least two different techniques should be used in order to solve sampling variation.[24] In the present study,

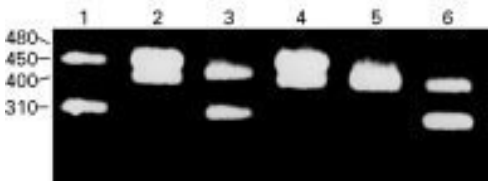

Figure 1: PGC genotypes detected after PCR amplification.

H pylori infection was determined by culture, histological examination of biopsy samples from the gastric body and antrum, and enzyme linked immunosorbent assay (ELISA) with the GAP test IgG kit (Biometrica, California, USA).[25] Subjects who had *H pylori* infection identified by more than two tests were classified *H pylori* positive; those who did not have *H pylori* by all three tests were classified as negative.

STATISTICAL ANALYSIS

The significance of the association between peptic ulcer and pepsinogen RFLPs was tested using the χ^2 test, with significance assigned to values below $p<0.05$. Bonferroni's correction for multiple comparisons was made for the PGC alleles. The logit estimate of the common odds ratio was calculated using the method described by Woolf.[26]

The significance of the differences in serum PGA and PGC concentrations and the ratio of PGA to PGC were determined by Student's *t* test for unpaired samples, with significance assigned to values below $p<0.05$.

Results

RFLP FOR PGC

After amplification, PCR products were fractionated on agarose gels. Four alleles could be resolved after ethidium bromide staining and UV irradiation. The sizes of these alleles were 480 (allele 1), 450 (allele 2), 400 (allele 3), and 310 bp (allele 4) (fig 1). In contrast, four EcoRI restriction fragments (20, 5.7, 3.6, and 3.5 kb) were observed among the genotypes, of which two were shown to be polymorphic (3.6 and 3.5 kb) by Southern analysis. A comparison of the PGC alleles detected by PCR and Southern blotting revealed that the 480, 450, and 400 bp alleles amplified by PCR corresponded to the larger EcoRI allele of 3.6 kb, and that the 310 bp PCR amplified allele corresponded to the smaller EcoRI allele of 3.5 kb (fig 2). Individual 3 is homozygous for the larger fragment allele (3.6 kb), individuals 2, 4, and 5 are homozygous for the smaller fragment allele (3.5 kb), and individuals 1, 6, and 7 are heterozygous for the RFLP. PGC genotypes were detected by PCR amplification. The 310 bp allele (allele 4) corresponds to the small fragment allele (3.5 kb), and the 480 (allele 1), 450 (allele 2), and 400 bp allele (allele 3) correspond to the larger fragment allele (3.6 kb). Individuals 2, 4, and 5 are homozygous for allele 4, individual 1 is heterozygous for alleles 2 and 4, individual 3 is homozygous for allele 2, individual 6 is heterozygous alleles for 1 and 4, and individual 7 is heterozygous for alleles 3 and 4.

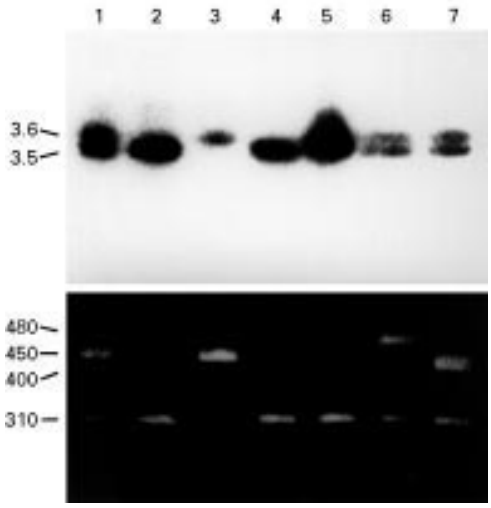

Figure 2: Comparison of PGC genotypes detected by Southern blotting (upper panel) and PCR (lower panel). Southern blotting of genomic DNA digested with EcoRI from seven unrelated individuals. The nitrocellulose blot was hybridised with a PGC cDNA probe (PGC301). The sizes of bands hybridising to the probe are indicated in kilobases.

DISTRIBUTION OF PGC-RFLP

Table 1 shows the distribution of genotypes for PGC-RFLP in patients with gastric or duodenal ulcer compared with controls, and table 2 shows the estimated allelic frequencies of PGC-RFLP. The increased frequency of allele 4 in patients with gastric body ulcer (0.450), compared with controls (0.268), was statistically significant ($\chi^2=9.92$, $p<0.005$), and it yielded an odds ratio of 2.23 (95% confidence limits, 1.35 to 3.68). The genotypes containing allele 4 (genotypes 1:4, 2:4, 3:4, and 4:4) were significantly more frequent in patients with gastric body ulcer (80.0%) than in controls (46.3%) or in patients with gastric angular or antral ulcer (42.9%). The relative risk of gastric body ulcer associated with genotype was estimated. The relative risk of gastric body ulcer associated with the presence of allele 4 (genotypes 1:4, 2:4, 3:4, and 4:4) compared with its absence (genotypes 1:1, 1:2, 1:3, 2:2, 2:3, and 3:3) was 4.63 (95% confidence limits, 2.02 to 10.62), and was statistically significant ($\chi^2=14.84$, $p<0.005$).

RELATION BETWEEN PGC-RFLP AND *H PYLORI* INFECTION

The prevalence of *H pylori* infection was 69.5% in healthy controls, 85.0% in patients with gastric body ulcer, 88.6% in patients with gastric angular or antral ulcer, and 91.4% in patients with duodenal ulcer. The relation between PGC-RFLP and *H pylori* infection was analysed. There were no significant differences in the allele frequencies of PGC-RFLP between the *H pylori* positive and *H pylori* negative groups in the controls, patients with gastric body ulcer, or gastric angular or antral ulcer (table 3). In both *H pylori* negative and *H pylori* positive cases, an increased frequency of allele 4 in patients with gastric body ulcer compared with controls was observed, and the genotypes containing allele 4 were significantly more frequent in patients with gastric body ulcer

TABLE 1 *The distribution of the PGC-RFLP in patients with gastric and duodenal ulcers compared with controls*

		Gastric ulcer			
RFLP pattern	*Controls*	*Body*	*Angulus*	*Antrum*	*Duodenal ulcer*
1:1	4	1	–	1	–
1:2	15	2	4	1	3
1:3	13	–	1	–	7
1:4	14	5	2	–	4
2:2	29	4	4	2	13
2:3	28	1	4	1	5
2:4	40	17	4	1	17
3:3	6	–	1	1	2
3:4	15	6	2	1	13
4:4	13	4	3	2	6
Total	177	40	25	10	70

TABLE 2 *Allele frequency of the PGC-RFLP*

		PGC-RFLP allele			
		1	*2*	*3*	*4*
Controls	n=177	0.141	0.398	0.192	0.268
Gastric ulcer					
Body	n=40	0.113	0.350	0.088	0.450*
Angulus	n=25	0.140	0.400	0.180	0.280
Antrum	n=10	0.150	0.350	0.200	0.300
Duodenal ulcer	n=70	0.100	0.364	0.207	0.329

*Significantly different from controls, χ^2=9.92, p<0.005.

TABLE 3 *Allele frequency of the PGC-RFLP with regard to H pylori infection*

			PGC RFLP allele			
			1	*2*	*3*	*4*
Controls	*H pylori* (+)	n=123	0.146	0.378	0.191	0.285
	H pylori (−)	n=54	0.130	0.444	0.194	0.232
Gastric ulcer						
Body	*H pylori* (+)	n=34	0.118	0.338	0.088	0.456*
	H pylori (−)	n=6	0.083	0.417	0.083	0.417
Angulus or antrum	*H pylori* (+)	n=31	0.145	0.387	0.177	0.290
	H pylori (−)	n=4	0.125	0.375	0.250	0.250

Significantly different from *H pylori* (+) controls, χ^2=7.17, p<0.01.

TABLE 4 *Distribution of PGC genotypes which possessed allele 4 in the controls and patients with gastric ulcer*

	Genotypes			
	H pylori (−)		*H pylori (+)*	
	Allele 4 (+)	*Allele 4 (−)*	*Allele 4 (+)*	*Allele 4 (−)*
Controls	22	32	60	63
Gastric ulcer				
Body	5*	1	27†	7
Angulus or antrum	2	2	13	18

*Significantly different from controls, χ^2=3.96, p<0.05.
†Significantly different from controls, χ^2=10.12, p<0.005.

TABLE 5 *Serum PGA and PGC concentrations (ng/ml) and the ratio of PGA to PGC (mean (SD))*

		Serum PGA	*Serum PGC*	*PGA:PGC ratio*
Controls				
H pylori (+)	n=123	50.1 (24.7)	18.4 (9.7)‡	3.1 (1.4)
H pylori (−)	n=54	45.4 (23.1)	8.2 (6.3)	6.2 (1.9)¶
Gastric body ulcer				
H pylori (+)	n=34	48.9 (28.1)	19.2 (11.6)	2.8 (1.2)
H pylori (−)	n=6	38.3 (16.1)	12.3 (10.8)	3.0 (2.1)
Gastric angular or antral ulcer				
H pylori (+)	n=31	61.7 (27.8)*	19.9 (11.1)§	3.4 (1.3)
H pylori (−)	n=4	47.6 (2.2)	7.4 (1.3)	6.6 (1.1)**
Duodenal ulcer				
H pylori (+)	n=64	75.5 (18.2)†	19.0 (11.2)	4.4 (1.6)
H pylori (−)	n=6	54.4 (16.4)	13.2 (8.2)	5.0 (1.8)

*Significantly different from *H pylori* (−) patients with gastric angular or antral ulcer (p<0.01).
†Significantly different from *H pylori* (−) patients with duodenal ulcer (p<0.025).
‡Significantly different from *H pylori* (−) controls (p<0.005).
§Significantly different from *H pylori* (−) patients with gastric angular or antral ulcer (p<0.005).
¶Significantly different from *H pylori* (+) controls (p<0.005).
**Significantly different from *H pylori* (+) patients with gastric angular or antral ulcer (p<0.01).

(83.3% in *H pylori* negative, 79.4% in *H pylori* positive cases) than in controls (40.7% in *H pylori* negative cases, χ^2=3.96, p<0.05; 48.8% in *H pylori* positive cases, χ^2=10.12; p<0.005) (table 4).

SERUM PGA AND PGC CONCENTRATIONS AND THE RATIO OF PGA TO PGC

Table 5 presents serum PGA and PGC concentrations and the ratio PGA:PGC. There were no significant differences in serum PGA and PGC concentrations and the PGA:PGC ratio among the genotypes of PGC-RFLP in the *H pylori* negative and positive controls. Serum PGA concentrations were higher in *H pylori* positive groups than in *H pylori* negative groups. The increases in serum PGA concentrations were statistically significant in both patients with gastric angular or antral ulcer (p<0.01) and duodenal ulcer (p<0.025). Serum PGC concentrations were also higher in *H pylori* positive groups than in *H pylori* negative groups. The increases in serum PGC concentrations were statistically significant in controls (p<0.005) and patients with gastric angular or antral ulcer (p<0.005). The PGA:PGC ratios were higher in *H pylori* negative groups than in *H pylori* positive groups. The increases in the PGA:PGC ratios were statistically significant in controls (p<0.005) and patients with gastric angular or antral ulcer (p<0.01).

Discussion

PCR analysis revealed four alleles for the PGC gene. Alleles which could not be resolved by Southern blotting were resolved on conventional agarose gels by PCR. Three alleles (alleles 1, 2, and 3), which had previously been classified as the upper allele of the insertion-deletion PGC-RFLP by Southern blotting,[18] were discriminated. Our data indicate an association between genetic polymorphisms at the PGC gene locus and gastric body ulcer. The frequency of allele 4 in the PGC-RFLP of patients with gastric body ulcer (0.450) was significantly higher than that of controls (0.268). The odds ratio of gastric body ulcer associated with the presence of allele 4 of PGC-RFLP was 2.23, and this was statistically significant. The genotypes containing allele 4 (genotypes, 1:4, 2:4, 3:4, and 4:4) were significantly more frequent in patients with gastric body ulcer (80.0%) than in controls (46.3%) or patients with gastric angular or antral ulcer (42.9%). These results suggest that the PGC gene locus contributes to susceptibility to gastric body ulcer. The relative risk associated with the presence of allele 4 as compared with the absence of allele 4 was 4.63 (95% confidence limits, 2.02 to 10.62) and was statistically significant (χ^2=14.84, p<0.005).

Our data also indicate the genetic heterogeneity of gastric ulcer disease. The frequency of allele 4 in PGC-RFLP was significantly higher in patients with gastric body ulcer than in those with gastric angular or antral ulcer. These findings indicate that there are differences in the genetic aetiology of gastric body ulcer and gastric angular or antral ulcer, and this suggests

that PGC-RFLP is a useful genetic marker with which to distinguish these genetic differences. Peptic ulcer is a multifactorial inherited disease. Difficulties have been encountered in attempts to identify the genes involved in the pathogenesis of the disease, such as problems with phenotype definition, identifying informative families for genetic studies, and the heterogeneity of the disease. 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 been demonstrated in peptic ulcer disease. Doll and Kellock demonstrated the independent segregation of gastric and duodenal ulcers. They also concluded that genetic factors alone, without environmental interactions, could account for this segregation.[27] Other subgroups have been defined by identifying pathophysiological disturbances, such as gastric hypersecretion, an increased rate of gastric emptying, or increased gastrin release, and combining these abnormalities with genetic markers.[28–30] In this study, the PGA:PGC ratio was significantly lower in patients with gastric body ulcer than in patients with duodenal ulcer and in patients with angular or antral ulcer. Samloff *et al* reported that a low PGA:PGC ratio is a subclinical marker of atrophic gastritis.[31] In addition, Tatsuta and Okuda demonstrated that ulcers are more proximal when atrophic gastritis is more severe.[32] These findings suggest that the pathophysiological factors are different between gastric body ulcer and gastric angular or antral ulcer.

Although the pathogenic mechanisms of *H pylori* infection are not known, *H pylori* infection is strongly associated with gastric ulcer. In this study the prevalence of *H pylori* infection was significantly higher in patients with gastric ulcer than in the healthy controls. In this study we also examined the relation between the genetic association of PGC polymorphisms with gastric body ulcer and *H pylori* infection. There were no significant differences in the allelic frequencies of PGC-RFLP between *H pylori* positive and *H pylori* negative groups in controls and in patients with gastric body ulcer. In both *H pylori* negative and *H pylori* positive cases, an increased frequency of allele 4 in patients with gastric body ulcer compared with controls was observed, and the genotypes containing allele 4 were significantly more frequent in patients with gastric body ulcer than in controls. These results indicate that the association between genetic polymorphisms of the PGC gene and gastric body ulcer is not associated with *H pylori* infection.

Previous studies on PGC genomic clones suggested that RFLP involves a 100 bp insertion or deletion of an intron sequence between exons 7 and 8.[17] Gene polymorphisms caused by the presence or absence of sequence insertions have been described in several mammalian genes, for instance in the 5' flanking region of the human insulin receptor gene and the 5' flanking region of the rat prolactin gene.[33 34] In both instances, sequence analysis revealed that DNA insertions consisted of repetitive elements. Our previous analysis of PGC-RFLP indicated that a dinucleotide repeat sequence, $(TA)_n(CA)_n$ is involved.[19] It is unlikely that the rearrangement of the gene reflects a change in the amino acid sequence of PGC itself. However, the possibility cannot be excluded, as differential PGC pre-mRNA splicing depends on the presence or absence of the insertion, and part of this sequence might be present in the mature RNA, thus modifying its stability or giving rise to the presence of an additional peptide inside the protein, and consequently altering its secretion or stability. In this study, PGA and PGC concentrations were quite different between *H pylori* negative and *H pylori* positive cases; however, there was no significant difference in serum PGA and PGC concentrations and the PGA:PGC ratio among the various PGC-RFLP genotypes in both *H pylori* negative and *H pylori* positive controls. We cannot conclude from this study that RFLPs are responsible for a PGC abnormality which is significantly associated with the development of gastric body ulcer. The RFLP is more likely to be linked to a disequilibrium with the causative gene for gastric body ulcer. Although the candidate gene approach has the advantage that sequences can be directly related to a known physiological function, it is restricted to characterised genes. As most genes relating to peptic ulcer are still unknown, linkage analysis with random markers will be necessary for studying the genetic component of peptic ulcer disease. The PGC gene has been localised to human chromosome 6p21.1-pter by analysis of mouse × human somatic cell hybrids.[17] Recent linkage analysis demonstrated that the PGC gene is 22 cM proximal to HLA-DPB, between D6S5 and D6S4, at a distance of 4.5 and 13.1 cM.[19] Further molecular biological studies using RFLP markers for this chromosome region will clarify whether PGC-RFLP is linked to a disequilibrium of the causative genetic variations for gastric body ulcer.

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