

Effect of a Specific Cyclooxygenase-Gene Polymorphism (A-842G/C50T) on the Occurrence of Peptic Ulcer Hemorrhage

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Abstract Cyclooxygenases (COX) catalyze the conversion of arachidonic acid to prostaglandins (PGs). COX-inhibiting drugs, such as nonsteroidal anti-inflammatory drugs (NSAIDs), increase the risk for peptic ulcer disease. As a corollary, COX gene polymorphisms could be important in the pathogenesis of peptic ulcer disease because these affect prostaglandin formation and impair its protective effect at the level of the gastric mucosa. This study was designed to investigate the association between the functional single-nucleotide polymorphism, A-842G/C50T, in the COX-1 gene and peptic ulcer bleeding. We obtained DNA samples from 106 patients who underwent upper gastrointestinal endoscopy because of bleeding peptic ulcer disease and from 88 healthy control subjects. Genetic polymorphism in A-842G/C50T was determined by PCR followed by restriction-fragment-length-polymorphism analysis. Adjusted logistic regression analysis was performed to evaluate the associations. Risk factors associated with peptic ulcer bleeding were male gender (odds ratio, 4.78; 95% confidence interval, 2.6–8.8) and NSAID/aspirin-use (odds ratio, 38.39; 95% confidence interval, 14.2–103.6). The A-842G/C50T

heterozygote was less frequent in peptic ulcer bleeding ($n = 7$) compared with healthy control subjects ($n = 11$). The adjusted risk for peptic ulcer bleeding among individuals who were heterozygote for the A-842G/C50T polymorphism was 0.75 (range, 0.19–3.01) compared with wild type. The COX-1 A-842G/C50T SNP does not influence the risk for developing peptic ulcer bleeding.

Keywords Cyclooxygenase-1 · Genetics · *Helicobacter pylori* · Peptic ulcer disease · Hemorrhage

Infection with *Helicobacter pylori* and use of nonsteroidal anti-inflammatory drugs (NSAIDs) have been identified as major contributing factors to the development of peptic ulcer disease [1]. NSAIDs are well known to induce gastric mucosal damage, including bleeding, ulceration, and perforation [2]. These effects are related to the inhibition of the enzyme cyclooxygenase (COX), which catalyses the formation of prostaglandin from arachidonic acid [3]. Prostaglandin synthesis holds the key to gastric mucosal integrity because it, among others, inhibits acid secretion, stimulates mucus and bicarbonate secretion, inhibits apoptosis, and increases mucosal blood flow. COX exists in two isoforms, COX-1 and COX-2, but in the gastric mucosa there is ubiquitous expression of COX-1 where it contributes in large part to the prostaglandin synthesis [4]. The ulcerogenic effects of NSAIDs are believed to be the consequence of inhibition of COX-1, as the development of specific COX-2 inhibitors has demonstrated. For example, the use of a COX-1 inhibitor, such as naproxen, is associated with at least a twofold higher risk of complicated peptic ulcer disease compared with a COX-2 inhibitor, such as rofecoxib [5].

Functional COX-1 gene polymorphisms could be important in the pathogenesis of peptic ulcer because these inhibit prostaglandin (PG) formation and consequently impair

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its protective effect at the level of the gastric mucosa. The COX-1 gene is composed of 11 exons spanning a length of 22 kilobases (kb) on chromosome 9 and encodes for a 576 amino acids long protein [6]. There seems to be appreciable genetic diversity within the COX-1 locus, and at least nine different single nuclear polymorphisms (SNP) have been identified so far [7, 8]. Specifically, two SNP A-842G and C50T, which are in complete linkage disequilibrium, seem to have functional consequences. Heterozygotes for A-842G/C50T exhibited increased sensitivity to acetylsalicylic acid and had much lower prostaglandin synthesis capacity compared with the wild type [7]. This might suggest that A-842G/C50T carriers run at a higher risk of ulcerogenic side effects of NSAIDs because of their decreased ability to catalyze the formation of prostaglandin from arachidonic acid.

The objective of this study was to investigate the association between the COX-1 A-842G/C50T SNP, NSAIDs use, and peptic ulcer bleeding.

Materials and methods

Patients who underwent an upper gastrointestinal endoscopy in the Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands, from January 1999 until June 2002, and presented with bleeding peptic ulcer disease were invited to take part in our study. We retrieved the data of 233 eligible patients and sent a structured questionnaire and an invitation to participate after discharge from the hospital. The inclusion criteria were as follows: older than aged 18 years, and a diagnosis of active peptic ulcer disease as established by endoscopy. The location, size, and activity of the ulcer(s) were recorded on a standard form.

We diagnosed a gastric ulcer (GU) or duodenal ulcer (DU) when there was a break in the mucosa >0.5 cm in diameter. We only included active bleeding ulcers, whereas malignant ulcers were excluded. Active ulcers were diagnosed in case of edematous mucosa around the ulcer crater. Bleeding ulcers were diagnosed when patients presented with 1) hematemesis, and 2) had an ulcer and/or ulcer that was actively bleeding, or 3) when there were stigmata of recent bleeding, such as a visible vessel or clot attached to the base of the ulcer and/or melena in presence of an ulcer [9]. We were able to recruit data and DNA samples from 106 patients (48%) with a bleeding GU, DU, or both.

The control group consisting of 88 healthy subjects was recruited by advertisement in a local newspaper in June 2002. Patients and healthy control subjects were from the same hospital catchments area in The Netherlands, ensuring that the populations were ethnically homogeneous. Endoscopy was not performed in the control groups because, apart from obvious ethical implications, the probability of finding an

actively bleeding peptic ulcer in patients without apparent symptoms is low [10].

Data assessment

A structured questionnaire was used to collect information about medication, aspirin or NSAID-use (frequency, dose), comorbidity, and intoxications (smoking). A blood sample was drawn from all patients and healthy control subjects. The study was approved by the local medical ethical review committee, and all subjects gave their written, informed consent.

Genotyping

Genomic DNA was extracted from 300 μ l of EDTA blood, using the Puregene isolation Kit[®] (Gentra Systems, Minneapolis, MN). The A-842G/C50T COX-1 polymorphism was studied by using polymerase chain reaction, followed by restriction fragment length polymorphism (RFLP). A DNA fragment of 484 bp containing the C50T polymorphism was amplified using the forward primer 5'-CCAGCAGCCGCGCCATGAG-3' and reverse primer 5'-ACAGGTGAGGGGATGGATAG-3'. The 50 μ l of PCR reaction mixture contained 10 mM of Tris-HCL (pH 9.0), 50 mM of KCL, 0.1% TRITON 1.5 mM of MgCl₂, 0.25 mM of dNTPs, 3 U of Taq-DNA-polymerase, 10 pmol forward primer, 10 pmol reverse primer, and 200 ng of genomic DNA. PCR conditions on the Biorad iCycler were 95°C for 4 min, followed by 37 cycles of 95°C for 30 sec, 63°C for 30 sec, and 72°C for 30 sec, and finally an elongation step at 72°C for 5 min. The first 484 bp PCR fragment was used as a template in the second PCR amplifying a 178 bp fragment using the forward primers 5'-CCAGCAGCCGCGCCATGAG 3' and reverse primer 5'-CGAGCAGGACGGGGAGCTGC-3'. The bold **T** in the reverse primer introduces an alteration in the 178 bp product, so a second Aci I restriction site disappears. PCR conditions were the same as mentioned previously with minor modifications. For the 178 bp PCR, 1.0 mM MgCl₂ was used with an annealing temperature of 60°C, and we also added 2% DMSO. The 178 bp product was subjected to digestion for 16 hr at 37°C with Aci I. Digested samples were run on a 3% agarose gel (RESponse; Biozym) stained with ethidiumbromide and visualized with an UV illuminator. The wild type showed fragments of 158 bp + 20 bp. Heterozygotes for C50T demonstrated of 178 bp + 158 bp + 20 bp, whereas homozygotes had the undigested 178 bp fragment. In all samples investigated, the A-842G was in complete linkage disequilibrium with the C50T polymorphism, and we further denote this polymorphism as A-842G/C50T.

Table 1 Baseline characteristics

	Patients with bleeding peptic ulcer (<i>n</i> = 106)	Healthy control subjects (<i>n</i> = 88)	<i>P</i> value
Mean age (SD)	62.5 (12.9)	62 (8.5)	0.78
Male gender	72 (68)	27 (31)	<0.01
<i>H. pylori</i> infected	59 (56)	41 (47)	0.2
Currently smoking	35 (33)	55 (63)	<0.01
NSAID/ASA	74 (70)	5 (6)	<0.01

Note. NSAID, Nonsteroidal anti-inflammatory drug; ASA, Acetylsalicylic acid; SD, Standard deviation. Data are numbers with percentages in parentheses unless otherwise indicated.

Helicobacter pylori diagnosis

The presence of *H. pylori* infection in patients and controls was determined by histologic examination of biopsies taken at the antrum and corpus of the stomach during endoscopy procedures and/or serologic testing using a commercial IgG ELISA kit (Pyloriset, Orion Diagnostics).

Data analysis

Frequency tables were calculated to obtain information about the occurrence of the different risk factors and polymorphic variants between the three groups. The Hardy-Weinberg equilibrium was calculated to describe the relationship between gene frequency and genotype frequency. Differences in demographics across patients and controls were evaluated with Chi-square testing. Statistical analysis was performed calculating unadjusted odds ratios and its 95% confidence intervals for the occurrence of bleeding peptic ulcer disease, the different polymorphisms, and risk factors. Adjusted odds ratios were calculated with the use of a logistic regression model after adjustment for baseline demographic characteristics, coexisting diseases, and medication use. In this model, we simultaneously included age, gender, NSAID-use, smoking, *H. pylori* infection and A-842G/C50T polymorphism. Then, the location (DU/GU) and number of ulcers between patients with and without the A-842G/C50T polymorphism were compared.

Based on the reported frequency of the A-842G/C50T polymorphism of 18% in the healthy population by Halushka *et al.* [7], we calculated that to detect a difference of 20% it was necessary to study 88 peptic ulcer patients and 88 controls to obtain 0.8 power and alpha values of 0.05.

Results

Demographic and clinical characteristics

We performed genotyping for COX-1 A-842G/C50T in a total of 194 white individuals: 106 peptic ulcer patients, and 88 healthy control subjects. Sixty-one bleeding peptic ulcer patients had DU, whereas 45 had GU. In 28 patients (26%),

more than one peptic ulcer was found during endoscopy. Subjects in the patient group and control group did match for age (Table 1).

COX-1 enzyme polymorphism

On RFLP analysis, 18 of 194 (9%) tested heterozygote and none were homozygote for the A-842G/C50T polymorphism. The COX-1 snp allele frequency was 7% in the peptic ulcer group and 13% in the healthy control subjects (Table 2). Analysis demonstrated that five of the seven patients with the COX-1 SNP had used acetylsalicylic acid, whereas the other two patients had not used NSAIDs or aspirin. None of the 11 control subjects with the COX polymorphism were using NSAIDs, nor had a history of chronic NSAID or aspirin use. The genotype frequencies were in Hardy-Weinberg equilibrium in both groups.

The unadjusted risk for peptic ulcer hemorrhage among COX-1 SNP carriers was 0.49 (95% confidence interval (CI), 0.2–1.3) compared with wild type controls (Table 3). Male gender (odds ratio, 4.78; 95% CI, 2.6–8.8) and NSAID/aspirin-use (odds ratio, 38.39; 95% CI, 14.2–103.6) were statistically significant associated with peptic ulcer bleeding, whereas current smoking and infection with *H. pylori* were not (Table 3).

A-842G/C50T heterozygosity in the ulcer group

All seven patients with peptic ulcer hemorrhage who were heterozygous for the A-842G/C50T polymorphism had a duodenal ulcer (*P* = 0.01). Four of seven patients were using aspirin and were *H. pylori*-positive. The other three patients were not using NSAIDs or aspirin nor were infected with

Table 2 Frequencies of the COX-1 A-842G/C50T polymorphism

A-842G/C50T	Patients with peptic ulcer (<i>n</i> = 106)	Healthy control subjects (<i>n</i> = 88)
Wild type	99 (93)	77 (87)
Heterozygous	7 (7)	11 (13)
Homozygous	0 (0)	0 (0)

Note. Data are numbers with percentages in parentheses.

Table 3 Association between peptic ulcer bleeding and important risk factors

	Unadjusted odds ratio (95% confidence intervals)	Adjusted odds ratio ^a (95% confidence intervals)
A-842G/C50T	0.5 (0.18–1.34)	0.75 (0.19–3.01)
Male gender	4.78 (2.6–8.8)	4.74 (2.11–10.64)
<i>H. pylori</i> infected	1.44 (0.82–2.54)	1.60 (0.71–3.6)
Currently smoking	0.3 (0.17–0.54)	0.60 (0.27–1.36)
NSAIDs/ASA	38.37 (14.22–103.6)	33.05 (11.32–96.49)

Note. NSAID, Nonsteroidal anti-inflammatory drug; ASA, Acetylsalicylic acid; SD, Standard deviation.

^aAdjusted for gender, age, smoking, NSAID/aspirin use, and *Helicobacter pylori* infection.

H. pylori. There was no particular enrichment of the COX-1 SNP in the group of patients with two or more ulcers.

Discussion

We examined the association between the functional A-842G/C50T SNP in the COX-1 enzyme and peptic ulcer bleeding. Our data showed an inverse association between the prevalence of the A-842G/C50T polymorphism and bleeding peptic ulcer disease in our white population. Individuals with the COX-1 polymorphism less often had a bleeding peptic ulcer compared with those without the polymorphism, but this association was not statistically significant. Once again, our study confirms that use of NSAIDs and male gender were other major risk factors for bleeding peptic ulcer disease, whereas in our model *H. pylori* infection and smoking seemed to be less important. The results we obtained were unexpected and suggest that the relationship between COX enzyme activity and bleeding peptic ulcer disease is more complex than previously anticipated.

Because previous experimental data suggested that A-842G/C50T is associated with a loss of COX-1 enzyme function [7], we reasoned that a polymorphism would affect gastric cytoprotection and lead to peptic ulceration with or without complications, such as bleeding. On the other hand, our results are more in line with findings from earlier animal studies in which gene disruption experiments have assessed the biologic roles of the COX-1 and COX-2 enzymes [10, 11]. Remarkably COX-1 enzyme deficient mice had no gastric pathology and were resistant to indomethacin (an inhibitor of PG-synthesis)-induced gastric ulceration. There was a 99% reduction in gastric PGE₂ levels, but none of the mice developed peptic ulcers. Thus, the absence of the COX-1 enzyme alone is not sufficient to cause stomach ulceration in mice. The lack of gastric ulceration in COX-1 deficient mice might be attributed to compensatory mechanisms by other enzymes, and in these mice, the COX-2

enzyme seems to be the alternative source for PG synthesis. In contrast to COX-1 deficient mice, COX-2 deficient mice developed more dramatic phenotypic changes [11], such as renal dysplasia, cardiac fibrosis, and female infertility.

Only few studies have studied the physiologic consequences of genetic variation in COX-1 [7, 8]. The results from the study by Halushka *et al.* showed that A-842G/C50T heterozygosity was associated with decreased PGH₂ synthesis after acetylsalicylic acid administration. PGH₂ is converted from arachidonic acid by the action of COX-1. However, they were unable to detect statistical significant differences in the amount of COX-1 enzyme expressed by platelets between the A-842G/C50T polymorphism and the wild type. There also was no statistically difference between heterozygotes and wild types for arachidonic acid induced PGF_{2a} synthesis, which is a marker for the PGH₂ production. Normally, COX-1 is only active at high concentrations of arachidonic acid. Lower levels of COX-1 would be more readily inhibited by a given concentration of acetylsalicylic acid, resulting in lower amounts of PGH₂ produced after an arachidonic acid challenge. Thus, individuals with the A-842G/C50T polymorphism may possess a decreased ability to metabolize arachidonic acid by COX-1.

A-842G is located in the promoter of COX-1, and the G allele creates a theoretic AP2 transcription factor site that might translate into a different expression of the gene. The C50T variant codes for a nonsynonymous substitution (Pro17Leu) 6 amino acids proximal to the signal peptide cleavage site and might translate into a miscompartmentalization of the protein. Halushka *et al.* found that the haplotype of SNPs A-842G and Pro17Leu is associated with an increased inhibition of PGH₂ synthesis by aspirin compared with the wild type [7]. This was not dependent on reduced expression of COX-1 or an alternative compartmentalization of COX-1. Importantly, the possible subtle reduced expression of COX-1, as a consequence of the polymorphisms, did not translate into important changes in TXB₂ production. This may suggest a marginal effect of 842G/50T heterozygotes on COX-1-dependent platelet TXA₂ biosynthesis. The prevalence of A-842G/C50T heterozygosity in the control group was comparable with the study of Halushka *et al.*: they found 19% heterozygotes, and homozygotes were predicted to occur as approximately 1% of the white population, which was consistent with our findings [7].

Recently Fries *et al.* provided evidence that COX-1 gene variability has indeed functional consequences. In vivo and in vitro studies with cells from C50T COX1 carriers showed that “selective” COX-2 inhibitors, rofecoxib and celecoxib, led to reduced COX-1 inhibition compared with wild type [12]. Thus, the possible influences of COX-1 polymorphisms on prostanoid biosynthesis and degree of inhibition by COX inhibitors are complex and not completely understood.

One potential limitation of this study is that we did not exclude active peptic ulcer disease in our control population. We anticipated that the probability of finding actively bleeding peptic ulcer disease in patients without apparent symptoms is low. Patients with peptic ulcers in the active stage usually experiences upper gastrointestinal symptoms [13]. The results from a study in Norway showed that only 1 percent of asymptomatic subjects had nonbleeding peptic ulcer disease [14]. Subjects using aspirin or NSAIDs have a higher risk for erosions or ulcers. Laine and colleagues showed in a recent study that the incidence of peptic ulcer disease was 4.9% and 14.3% in aspirin and NSAID users respectively [15]. Most of these ulcers are rather insignificant erosions, in contrast to symptomatic bleeding peptic ulcers in our study group. If we assume that all NSAIDs/aspirin users in the control groups had bleeding peptic ulcer disease the detected differences for the COX-1 SNP frequency would increase as only one control subject with the COX polymorphism was using a NSAID.

In conclusion, this study showed that individuals with a COX-1 A-842G/C50T polymorphism have a lower, but non-significant, risk for a bleeding peptic ulcer bleeding compared with wild type.

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References

1. Wolfe MM, Lichtenstein DR, Singh G (1999) Gastrointestinal toxicity of nonsteroidal antiinflammatory drugs. *N Engl J Med* 340:1888–1899
2. Garcia Rodriguez LA, Jick H (1994) Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs. *Lancet* 343:769–772
3. McCarthy DM (1995) Mechanisms of mucosal injury and healing: the role of non-steroidal anti-inflammatory drugs. *Scand J Gastroenterol Suppl* 208:24–29
4. Morita I (2002) Distinct functions of COX-1 and COX-2. *Prostaglandins Other Lipid Mediat* 68–69:165–175
5. Bombardier C, Laine L, Reicin A, *et al.* (2000) Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. *N Engl J Med* 343:1520–1528
6. Kraemer SA, Meade EA, DeWitt DL (1992) Prostaglandin endoperoxide synthase gene structure: identification of the transcriptional start site and 5'-flanking regulatory sequences. *Arch Biochem Biophys* 293:391–400
7. Halushka MK, Walker LP, Halushka PV (2003) Genetic variation in cyclooxygenase 1: effects on response to aspirin. *Clin Pharmacol Ther* 73:122–130
8. Ulrich CM, Bigler J, Sibert J, *et al.* (2002) Cyclooxygenase 1 (COX1) polymorphisms in African-American and Caucasian populations. *Hum Mutat* 20:409–410
9. Silverstein FE, Faich G, Goldstein JL *et al.* (2000) Gastrointestinal toxicity with celecoxib vs nonsteroidal anti-inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: a randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. *JAMA* 284:1247–1255
10. Langenbach R, Morham SG, Tiano HF, *et al.* (1995) Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* 83:483–492
11. Dinchuk JE, Car BD, Focht RJ, *et al.* (1995) Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature* 378:406–409
12. Fries S, Grosser T, Price TS *et al.* (2006) Marked Interindividual Variability in the Response to Selective Inhibitors of Cyclooxygenase-2. *Gastroenterology* 130:55–64
13. Lu CL, Chang SS, Wang SS, Chang FY, Lee SD (2004) Silent peptic ulcer disease: frequency, factors leading to “silence,” and implications regarding the pathogenesis of visceral symptoms. *Gastrointest Endosc* 60:34–38
14. Bernersen B, Johnsen R, Straume B, Burhol PG, Jenssen TG, Stakkevold PA (1990) Towards a true prevalence of peptic ulcer: the Sorreisa gastrointestinal disorder study. *Gut* 31:989–992
15. Laine L, Maller ES, Yu C, Quan H, Simon T (2004) Ulcer formation with low-dose enteric-coated aspirin and the effect of COX-2 selective inhibition: a double-blind trial. *Gastroenterology* 127:395–402