

Localization of Cytochrome P4502E1 Enzyme in Normal and Cancerous Gastric Mucosa and Association with Its Genetic Polymorphism in Unoperated and Remnant Stomach

Shunji Kato¹, Zenya Naito², Noriko Matsuda¹, Hiroyuki Onodera¹,
Nobuyuki Sakurazawa¹, Naoyuki Yamashita¹, Yoshikazu Kanazawa¹, Itsuo Fujita¹,
Hiroshi Makino¹ and Eiji Uchida¹

¹Surgery for Organ Function and Biological Regulation, Graduate School of Medicine, Nippon Medical School

²Integrative Pathology, Graduate School of Medicine, Nippon Medical School

Abstract

Background: Exposure to nitroso compounds and the activity of cytochrome P450 2E1 (CYP2E1), an activation enzyme for these carcinogens, are important factors in gastric carcinogenesis. Here, we investigated the potential correlation between genetic variation in *CYP2E1* and its enzyme expression as detected with immunohistochemical (IHC) staining and cancer susceptibility in unoperated and remnant stomach.

Methods: Expression of CYP2E1 in the stomach (n=117) was detected with IHC staining using a polyclonal anti-CYP2E1 antibody. Interindividual variation in CYP2E1 enzyme activity was then compared with genetic polymorphisms in the transcriptional flanking region of the *CYP2E1* gene by restriction fragment length polymorphism (RFLP) detection using the Rsa I restriction enzyme. Genetic polymorphisms of Rsa I RFLP in *CYP2E1* were investigated in 499 patients with gastric cancer (466 unoperated stomachs and 33 remnant stomachs) and 553 control patients with benign gastroduodenal diseases.

Results: Mucosal IHC staining for CYP2E1 was stronger in areas of intestinal metaplasia, particularly in endocrine cells, which stained consistently and strongly. Expression of CYP2E1 enzyme in areas of IHC staining were confirmed with Western blot analysis and showed a significant association between the degree of staining and the *CYP2E1* genotype ($p < 0.01$) in cancer tissues and in the foveolar epithelium of normal gastric mucosa. No association between specific *CYP2E1* genotype and gastric cancer risk in the unoperated stomach was found in either the large study or the age- and gender-matched case-control study. However, the frequency of rare alleles (C1/C2 or C2/C2) was significantly higher in patients with cancer in the remnant stomach following gastrectomy than in controls subjects without cancer (odds ratio=2.8, 95% confidence interval=1.3–5.8) or those with primary gastric cancer (odds ratio=2.6, 95% confidence interval=1.3–5.5).

Correspondence to Shunji Kato, MD, Department of Surgery, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8603, Japan

E-mail: katoshun@nms.ac.jp

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Conclusions: *CYP2E1* genetic polymorphisms might correlate with CYP2E1 enzyme expression levels in normal and cancerous gastric tissues. These polymorphisms do not influence the development of primary stomach cancer but may do so in specific conditions, such as the remnant stomach after gastrectomy. (J Nippon Med Sch 2011; 78: 224–234)

Key words: genetic polymorphism, cytochrome p450 2E1/CYP2E1, gastric cancer susceptibility, immunohistochemistry, genotype-phenotype relationship, remnant stomach

Introduction

Many cancers, particularly those in the gastrointestinal tract, appear to be associated with environmental factors¹. Stomach cancer in men is most common in East Asia, South America, and Eastern Europe² and is one of the most common fatal diseases in Japan and certain regions of Asia and Eastern Europe^{3,4}. Gastric carcinogenesis is thought to be a multistep and multifactorial process⁵. In addition to infection with *Helicobacter pylori* (*H. pylori*), classified by the World Health Organization as a definite carcinogen⁶, and only 2–10% of stomach cancer will be occurred without *H. pylori* infection negative status⁷, other endogenous factors include bile and intestinal juice reflux-induced change in mucosal background in the remnant stomach after gastrectomy⁸. Important exogenous factors are thought to include exposure to nitroso compounds occurring via the diet, tobacco smoking, and occupation hazards. Most chemical carcinogens require metabolic activation to damage DNA. Nitroso compounds are typically activated by cytochrome P450 enzymes (CYP), especially CYP2E1^{9,10}. Although the presence of CYPs in the liver, lung, and nearly all other organs has been thoroughly investigated, little is known about CYP2E1 enzyme localization in the upper gastrointestinal tract. Given that the probable targets of nitroso compounds are the head and neck region, the esophagus, and the stomach, this paucity of information is important^{11,12}.

Recent advances in molecular techniques have revealed interindividual variations in various enzyme functions related to endogenous activation

for specific carcinogens. These variations are determined by genetic differences, including single nucleotide polymorphisms (SNPs). Thus, individual differences in carcinogen metabolism may be either inheritable or the result of sustained environmental exposure to chemical carcinogens, and individual differences in the expression of enzymes involved in carcinogen activation or detoxification may determine an individual's susceptibility to carcinogenesis. The major enzymes for the activation of chemical carcinogens are CYPs, including CYP2E1 for esophageal cancers and CYP1A1 for lung cancers¹³. Some of these CYPs have already been identified as key enzymes in the activation of chemical carcinogens, and SNP analysis has revealed the existence of genotype-phenotype relationships with regard to enzyme function¹⁴.

CYP2E1 enzyme activity has been associated with the risk of several cancers, mediated via genetic polymorphisms in the *CYP2E1* gene¹⁵. For example, a restriction fragment length polymorphism (RFLP) at a locus in the 5'-transcriptional region has been associated with an increase in *CYP2E1* gene expression¹⁶ and may influence the development of cancers of the upper gastrointestinal tract, including gastric cancer^{17,18}. Little is known about the expression and function of this enzyme or its localization in the gastric mucosa. The expression of *CYP2E1* messenger (m) RNA in the liver and peripheral blood lymphocytes has been reported^{19,20}, and some expression patterns show a slight correlation between the specific Rsa I RFLP genotype and mRNA expression levels in the liver¹⁹. However, because a specific antibody for the detection of CYP2E1 enzyme with immunohistochemical (IHC) staining has not been

Table 1 Incidence of gastric cancer by sex, *H. pylori* infection status, pepsinogen I level, and pepsinogen I/pepsinogen II ratio

		Non-cancer Controls	Primary Gastric Cancers	Odds Ratio (95% CI)
Sex	female	231	154	1.0
	male	322	310	1.5 (1.1–1.9)
<i>Helicobacter pylori</i>				
	negative	139	74	1.0
	positive	324	301	1.8 (1.3–2.5)
PG* I	>50 ng/mL	247	113	1.0
	≤50 ng/mL	196	163	1.8 (1.3–2.5)
PG* I/II ratio				
	>3.0	328	106	1.0
	≤3.0	115	168	4.5 (3.3–6.2)

*pepsinogen

available²¹ and this enzyme is only faintly detected with real-time polymerase chain reaction (PCR), little information regarding its detection in the esophagus or the stomach is available.

The objectives of the present study were threefold. First, we compared CYP2E1 enzyme expression levels in normal and cancerous parts of gastric mucosa with the genotype of the individual, as detected with SNP analysis, and enzyme expression, as determined with IHC staining. Second, we investigated the association between the CYP2E1 Rsa I polymorphism and the susceptibility of individuals to gastric cancer in an age- and gender-matched case-control study in unoperated stomachs. Third, given the putative role of acid neutralization in gastric carcinogenic cancer via its support of bacterial growth and the production of endogenous nitroso compounds by nitric oxide reduction activity, we investigated the association of CYP2E1 Rsa I polymorphisms with carcinogenesis in the remnant stomach after gastrectomy²².

Materials and Methods

Subjects

In the genetic polymorphism study, genotype-phenotype relationships for CYP2E1 in the normal and cancerous parts of the gastric mucosa as detected with CYP2E1 enzyme expression by means of IHC staining were determined in 117 patients with histologically confirmed gastric cancer.

In the case-control study, histological classifications were determined as follows: papillary and tubular adenocarcinomas were considered to be intestinal-type cancers, whereas poorly differentiated adenocarcinomas, signet-ring cell adenocarcinomas, and mucinous cell adenocarcinomas were considered to be diffuse-type cancers. Control subjects were 553 patients without cancer in whom benign gastric diseases, including gastric ulcers, duodenal ulcers, and gastritis, had been diagnosed by means of upper gastrointestinal endoscopy. The control subjects were matched according to age (within 5 years) and sex, with sex differences in gastric cancer incidence taken into consideration, giving a total of 290 cancer cases (181 men and 109 women) and 290 controls (181 men and 109 women) for age- and sex-matched case-control analysis. Next, 499 patients with gastric cancer (466 with primary gastric cancer and 33 with remnant cancer after gastrectomy), and 553 clinical control subjects were preliminarily analyzed to investigate the association between the specific CYP2E1 Rsa I RFLP genotype and risk of gastric cancer (**Table 1**). Tissue samples were obtained at Nippon Medical School Hospital (Tokyo Japan) from 1993 through 1999. This study was performed in accordance with the principles embodied in the Declaration of Helsinki, 1975, and informed consent was obtained from all subjects.

H. pylori Detection and the Pepsinogen Methods

Serum obtained with centrifugation was stored at

-80°C. Serum IgG antibody against *H. pylori* was assayed with enzyme-linked immunosorbent assay (ELISA) (Cobas Core, Anti-*H. pylori* EIA, Roche Molecular Diagnostics, Pleasanton, CA, USA). Briefly, after termination of the enzyme reaction, absorbance at 450 nm was measured, and the results are expressed quantitatively as an index according to the standard curve. Seropositivity was set as a value of ≥ 8 IU/mL according to preliminary experiments performed to optimize specificity and sensitivity and used to classify patients as negative or positive⁷. Levels of pepsinogen I and II were assayed with a radioimmunoassay kit (Dainabot Co., Ltd., Tokyo) according to a previously described method⁷. Detection limits with this kit were 0.1 to 160 ng/mL for pepsinogen I and 0.7 to 100 ng/mL for pepsinogen II.

Detection of CYP2E1 Enzyme with IHC Staining

After gastrectomy for the stomach cancer, 117 resected surgical specimens were fixed in 10% formaldehyde, embedded in paraffin, and sectioned into 3- μ m slices. The sections were stained with hematoxylin-eosin and observed under a light microscope. To detect CYP2E1 enzyme with IHC staining, an anti-human CYP2E1-402 polyclonal antibody was prepared with the human CYP2E1 14-amino-acid sequence EFPDPEKFKPEHFL (402-415). Rabbits were immunized with this antigen as a hapten, and the IgG fraction was purified from rabbit antiserum using protein A column purification and used as antibodies. The most homologous amino acid sequence to antigen 402 was human CYP2C10, with a homology of 62.5%, but this antigen did not cross-react with previously examined subtypes²³. Sections from each specimen region were immunohistochemically stained by means of the streptavidin-biotin complex method with anti-human CYP2E1-402 antibody and observed under a light microscope. Staining patterns in the mucosa of the corpus, body, and antrum of the stomach; normal regions of the mucosa; and areas of chronic active gastritis, intestinal metaplasia, and cancer were compared to those of the foveolar epithelium in normal gastric mucosa. To determine the genotype-phenotype relationship for the *CYP2E1* genetic

polymorphism and enzyme expression as detected with IHC in the gastric mucosa, the degree of staining in foveolar epithelial cells was compared with that in gastric endocrine cells, which stained consistently and strongly, as an internal control. This staining was classified as either "weak," i.e., negative or weakly positive compared with endocrine cells, or "strong," i.e., equal or strongly positive compared with endocrine cells. Expression of CYP2E1 enzyme in areas with IHC staining was confirmed with Western blot analysis in 5 cases, and specificity was confirmed with an absorption test using purified anti-human CYP2E1-402 immunogen.

RFLP Analysis of CYP2E1 Polymorphism

DNA extraction and RFLP analysis with PCR amplification were performed as previously described¹⁷. Briefly, DNA was extracted from the blood buffy coat. PCR was used to amplify the transcription regulatory region of *CYP2E1*, including the restriction enzyme recognition site for Rsa I, using appropriate primers (5'-CCAGTCGAGTCTACATTGTCA-3' and 5'-TTCATTCTGTCTTCTAACTGG-3'). RsaI enzyme digestion (5 units, Invitrogen Corp., Carlsbad, CA, USA) produced a 412-bp PCR product with 360-bp and 52-bp fragments. A second amplification using 5'-AAGTATAGAATGGCATAA CTC-3' and 5'-GTTCTTAATTCATAGGTTGCA-3' primers under the same PCR conditions yielded a 151-bp PCR product with 124-bp and 27-bp fragments after digestion. Homozygous individuals without an Rsa I site yielded a single 412-bp band after the first PCR and enzyme digestion and a 151-bp band after nested PCR (homozygous type C2 allele), whereas homozygous individuals with the Rsa I site yielded smaller 360-bp and 52-bp bands (homozygous type C1 allele) after the first PCR amplification and 124-bp (C1 allele) and 27-bp bands after nested PCR.

Statistical Analysis

The chi-square test, Fisher's exact test, and unadjusted odds ratios (ORs) with 95% confidence intervals (CIs) were determined with the SPSS (version 11.0) statistical analysis program for the Windows operating system (SPSS Inc., Tokyo).

Results

Subject Characteristics

In the genetic polymorphism study, the mean ages of male and female control subjects were 52.5 ± 14.6 years ($n=322$) and 49.8 ± 16.6 years ($n=231$), respectively, and those of male and female patients with gastric cancer were 63.5 ± 10.5 years ($n=310$) and 62.1 ± 12.7 years ($n=154$), respectively. The percentage of cases to controls was about 62% for men and 38% for women. Mean ages of men and women in the age- and gender-matched case-control subjects were 58.3 ± 10.9 years and 57.2 ± 13.9 years for the noncancer control cases and 58.6 ± 10.5 years and 57.0 ± 13.8 years, respectively, for the gastric cancer cases. Statistical differences in sex ($p<0.05$), *H. pylori* positivity ($p<0.05$), pepsinogen I level (≤ 50 ng/mL, $p<0.05$), and pepsinogen I/II ratio (≤ 3.0 , $p<0.01$) between the cases and controls were comparable to those in previous reports which were analyzed in smaller case-control numbers (Table 1). In the 33 patients with gastric cancer in the remnant stomach examined at our hospital over the same sample-collection period, excluding recurrent cancers, mean age was 66.9 ± 9.7 years ($n=29$) for men and 57.0 ± 11.7 years ($n=4$) for women. Reconstruction methods used after gastrectomy were Billroth I ($n=14$), Billroth II ($n=9$), Roux-en-Y ($n=1$), and unknown ($n=9$). The primary conditions requiring gastrectomy were gastric cancer in 13 cases and benign gastric conditions, including peptic ulcer, in 15 cases, and unknown in 5 cases.

IHC Staining for CYP2E1 in Normal Gastric Mucosa and Gastric Cancer

Localization of CYP2E1 enzyme in the stomach was determined by means of IHC staining with a polyclonal anti-CYP2E1-402 antibody in 117 patients with gastric cancer. Endocrine cells stained with chromogranin A were plentiful in areas of atrophic gastric mucosa and were consistently and strongly stained with anti-CYP2E1-402 (Fig. 1A–C). Goblet and Paneth cells in gastric glands were observed in areas of complete intestinal metaplastic gastritis, and the cytoplasm of goblet cells in this condition was

stained (Fig. 2D). In areas of normal mucosa, CYP2E1 IHC staining of the glandular and foveolar epithelium was weak, whereas areas of intestinal metaplasia and active inflammation stained more strongly (Fig. 2E). The degree of IHC staining in individual specimens was compared with that of endocrine cells as an internal control and was classified as “negative” (Fig. 3F: weak), “equal,” or “strongly positive” (Fig. 3G: strong). No statistical difference in the degree of IHC staining was observed among histological types. Furthermore, no staining of the foveolar epithelium was observed in comparison with metaplastic epithelium that was stained positively in the grade I type (Fig. 3H: weak). Interindividual variations in staining were also observed in the foveolar epithelium (Fig. 3I: strong).

Genotype of CYP2E1 Rsa I Polymorphism and Gastric Cancer Risk

Results of the CYP2E1 polymorphism analysis are shown in Table 2. Electrophoresis of CYP2E1 polymorphisms detected with restriction enzymes is shown in Figure 1. The gel picture for CYP2E1 represents 3 genotypes as RFLPs. In the Rsa I restriction enzyme analysis, the homozygous rare genotype (C2/C2) and heterozygous genotype (C1/C2), both of which were expected to be associated with stronger enzyme function, were not associated with the overall risk of gastric cancer (OR=1.1, 95% CI=0.8–1.4), even when analyzed by sex (male: OR=1.1, 95% CI=0.8–1.5; female: OR=1.0, 95% CI=0.6–1.5) or histologic type (intestinal type: OR=0.9, 95% CI=0.7–1.3; diffuse type: OR=1.2, 95% CI=0.8–1.6). Furthermore, no association between specific CYP2E1 genotypes and gastric cancer was found in age- and sex-matched control subjects (OR=1.0, 95% CI=0.7–1.4).

Association between IHC Staining and Rsa I Genetic Polymorphisms of CYP2E1

Interindividual variations were observed in the degree of IHC staining in the foveolar epithelium of normal gastric mucosa. This finding contrasts with that in endocrine cells, which showed consistent and strong staining. The degree of IHC staining was

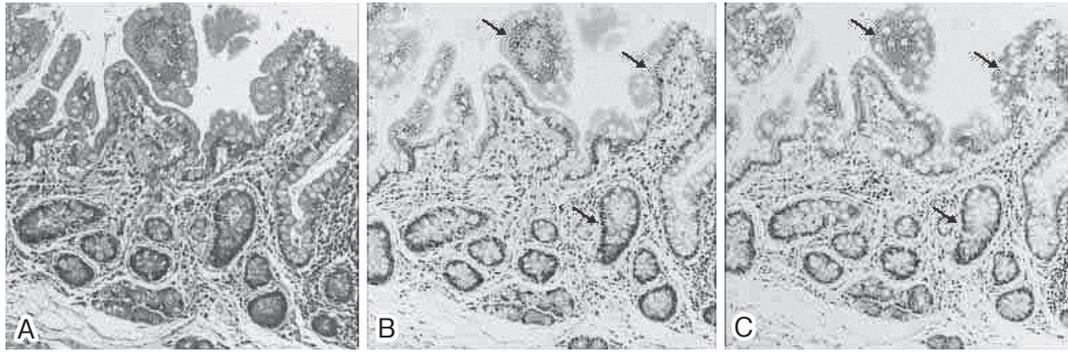


Fig. 1

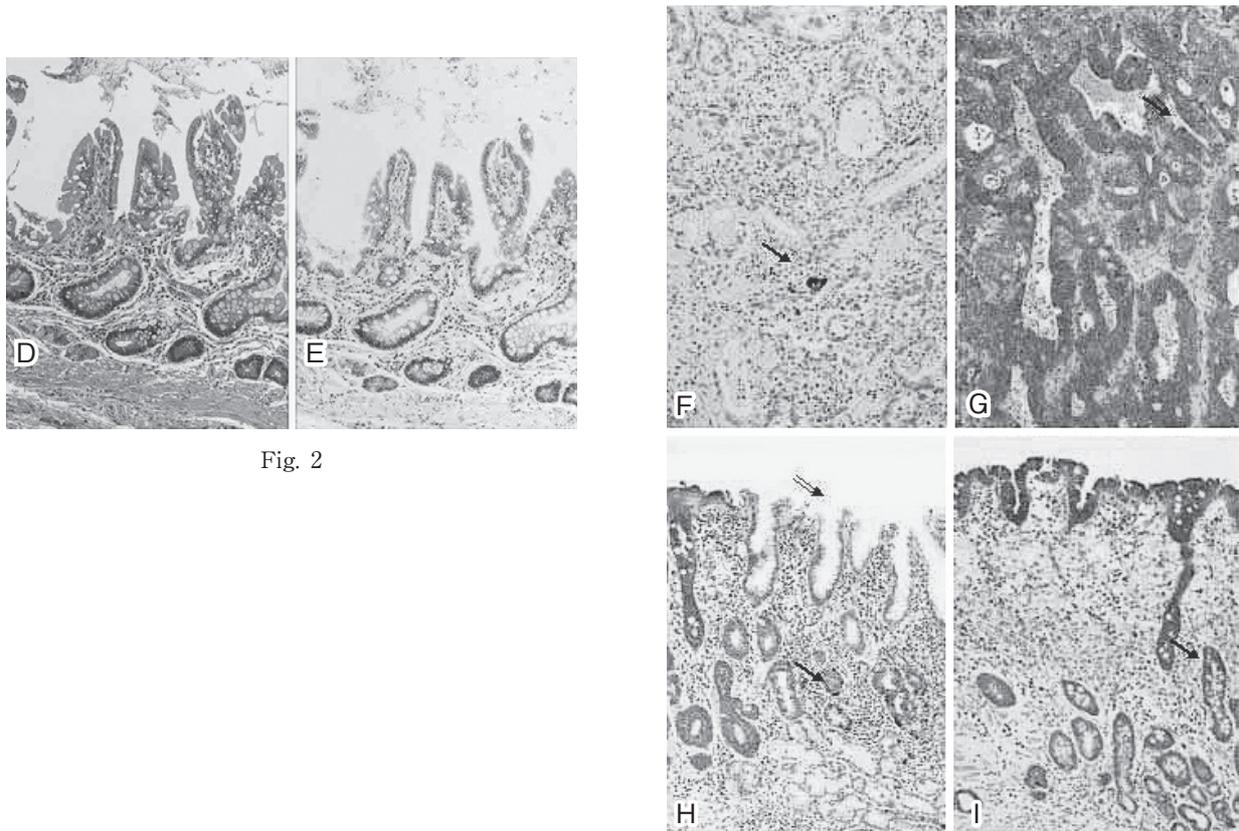


Fig. 2

Fig. 3

Fig. 1-3 (A-I) Hematoxylin and eosin, AB (Alcian-Blue)-PAS, and immunohistochemical staining of chromogranin A and CYP2E1 in gastric mucosa and cancer.

- A. Hematoxylin and eosin staining: Chronic atrophic gastritis shows atrophic changes of glands in the gastric mucosa, $\times 50$.
- B. Immunohistochemically, chromogranin A is stained in endocrine cells of the gastric mucosa. Endocrine cells are shown in the head of arrows, $\times 50$.
- C. Immunohistochemically, CYP2E1 is consistently and strongly observed (positive) in the cytoplasm of chromogranin A-positive endocrine cells and glandular epithelial cells, $\times 50$.
- D. Hematoxylin and eosin staining: Goblet and Paneth cells in gastric glands are observed in complete intestinal metaplastic gastritis, $\times 100$.
- E. The immunohistochemical staining for CYP2E1 in metaplastic foveolar epithelial cells shows a strong and diffuse expression pattern (positive) in the cytoplasm, $\times 100$.
- F. In "weak" (negative staining) cases, no immunohistochemical staining for CYP2E1 in carcinomas was observed, in contrast to the strongly positive staining of endocrine cells (**arrow**) and metaplastic foveolar epithelium, $\times 100$.
- G. "Strong" (equal or strongly positive) immunohistochemical staining for CYP2E1 in carcinomas shows a strong and diffuse expression pattern in all areas (positive), as does staining in endocrine cells (**arrow**), $\times 100$.
- H. In immunohistochemical staining for CYP2E1, no staining of the foveolar epithelium was observed, in contrast to the weakly positive staining in metaplastic epithelium (**double arrows**), but areas of intestinal metaplasia and active inflammatory regions stained more strongly (**arrow**), $\times 100$.
- I. Strong immunohistochemical staining for CYP2E1 in a foveolar epithelium shows a strong and diffuse expression pattern (positive), as does staining in endocrine cells (**arrow**), $\times 100$.

Table 2 Number of *CYP2E1* Rsa I genetic polymorphisms in all cases and in age- and gender matched case-control study cases

Subjects	Non-cancer Controls	Gastric Cancer Cases	Odds Ratio (95% CI)
All cases			
C1/C1	340	280	1.0
C1/C2 or C2/C2	213	186	1.1 (0.8-1.4)
Age- and gender-matched cases			
C1/C1	169	165	1.0
C1/C2 or C2/C2	112	109	1.0 (0.7-1.4)

Table 3 Immunohistochemical staining for *CYP2E1* enzyme and RsaI genetic polymorphism in gastric cancer tissues

Degree of Staining in Cancer Tissues* ¹	<i>CYP2E1</i> Genotype	
	C1/C1	C1/C2 or C2/C2* ²
Weak	27	5
Strong	26	23

*¹, Degree of staining was compared to that of endocrine cells, which was consistently strong.

*², p<0.01 for the chi-square test.

significantly associated with *CYP2E1* genotype in cancer tissues (p<0.01) and in the foveolar epithelium of normal gastric mucosa (p<0.05; **Tables 3 and 4**).

Remnant Stomach Cancer after Gastrectomy and *CYP2E1* Polymorphism

Cancer development in the remnant stomach occurred from 1 to 50 years after distal gastrectomy (mean, 18.5 ± 16.6 years). Mean time before cancer development was significantly longer in patients who underwent Billroth II reconstruction (35.7 ± 12.0 years, n=9) than in those who underwent Billroth I reconstruction (8.3 ± 9.0 years, n=14, p<0.05). In the *CYP2E1* genotype analysis, frequencies of the rare homozygous genotype (C2/C2) and the heterozygous genotype (C1/C2) were significantly higher than those of either the noncancer control cases or the cases of primary gastric cancer, the ORs of which were 2.8 (95% CI=1.3-5.8) and 2.6 (95% CI=1.3-5.5), respectively (**Table 5**). This tendency was more marked in patients who had undergone Billroth I reconstruction (Billroth I, OR=3.8, 95% CI=1.2-12.2, p<0.05; Billroth II, OR=1.9, 95% CI=0.5-7.1). Further genotype analysis classified by the primary gastric disease responsible for the gastrectomy showed that

8 of 13 patients with primary gastric cancer and 10 of 15 patients with primary peptic ulcer had the C2/C2 or C1/C2 genotype.

Discussion

To our knowledge, this study is the first to report a genotype-phenotype relationship for *CYP2E1* enzyme expression in human tissues. Because of the small sample size, no association was found between specific genotypes of the *CYP2E1* Rsa I polymorphism and this cancer risk.

CYP2E1 Enzyme Function and the Ability and Localization of Activation of Nitroso Compounds Related to Stomach Carcinogenesis

CYP2E1 is characterized by its ethanol inducibility and role in the metabolism of alcohols. It is also a key enzyme in the metabolic activation of various nitrosamines, in which it catalyzes the oxidation and DNA adduct formation of many small molecules having human carcinogenic potential^{9,24}. *CYP2E1* is mainly expressed in the liver, lung, and kidney, but previous reports have also shown, with IHC staining, weak expression in the stomach²¹. Here, we developed a new polyclonal antibody for the *CYP2E1* enzyme to determine its expression in the liver²³. However, we were also able to use this antibody to provide the first identification of this enzyme in gastric mucosa and gastric cancer tissues. This polyclonal antibody revealed that the *CYP2E1* enzyme is expressed in nearly all organs and that expression levels differ among organs⁹. This enzyme was strongly expressed in the liver in most subjects and slightly less strongly in areas of intestinal metaplasia and active inflammatory lesions of the

Table 4 Immunohistochemical staining for CYP2E1 enzyme and RsaI genetic polymorphisms in the foveolar epithelium of normal gastric mucosa

Staining in the Foveolar Epithelium of Normal Gastric Mucosa* ¹	CYP2E1 Genotype	
	C1/C1	C1/C2 or C2/C2* ²
Weak	26	5
Strong	27	23

*¹, Degree of staining was compared to that of endocrine cells, which was consistently strong.

*², $p < 0.01$ for the chi-square test.

Table 5 Remnant stomach cancer after gastrectomy and CYP2E1 genetic polymorphism

	Non-cancer Controls	Remnant Cancer Cases	Odds Ratio (95% CI)
C1/C1	340	12	1.0
C1/C2 or C2/C2	213	21	2.8 (1.3–5.8)* ¹
	Primary Gastric Cancer	Remnant Cancer Cases	Odds Ratio (95% CI)
C1/C1	280	12	1.0
C1/C2 or C2/C2	186	21	2.6 (1.3–5.5)* ¹

*¹, $p < 0.05$

stomach, as well as in endocrine cells in the gastric mucosa, which were consistently and strongly stained (**Fig. 1**). By using the same real-time PCR methods we had previously used for liver tissues¹⁹, we found that CYP2E1 mRNA was also expressed in the gastric mucosa, but at lower levels than in liver tissue.

This pattern of enzyme expression of CYP2E1 was readily confirmed with Western blot analysis (data not shown). In normal and cancerous parts of the gastric mucosa, the degree of IHC staining compared with the density of endocrine cells differed among individuals. This interindividual variation in enzyme activity was significantly associated with the CYP2E1 Rsa I RFLP genotype. This genotype-phenotype relationship may suggest that the exogenous dietary intake of N-nitroso compounds, or the endogenous production of nitrosamines by nitrate reductase in intestinal bacteria²⁴, enables their metabolic activation to direct carcinogens by CYP2E1 in the gastric mucosa and that CYP2E1 activity may influence the amount of carcinogenic substances present in individuals according to the genotype of the CYP2E1 Rsa I

polymorphism. Levels of mRNA in the liver or peripheral blood leukocytes are also reported to vary according to the CYP2E1 Rsa I RFLP^{19,20,25}.

CYP2E1 Genetic Polymorphism at the Specific Locus for Controlling Its Enzyme Functions and Stomach Cancer Susceptibility

In the present study, we have shown a possible relationship between CYP2E1 enzyme expression levels in the stomach and the CYP2E1 Rsa I genotype. Namely, expression levels of CYP2E1 were significantly higher in individuals with the C2/C2 or C1/C2 genotype than in those with the C1/C1 genotype. The initial identification of Rsa I RFLP in the CYP2E1 gene inspired a number of studies of possible functional differences in enzyme activity associated with specific SNPs and of potential associations with many kinds of cancers related to N-nitrosamine metabolism. These studies led to the identification of positive correlations^{15,26}, negative correlations²⁷, and inverted positive correlations^{18,28,29} between specific CYP2E1 Rsa I RFLP genotypes and the risk of several kinds of cancer, including nasopharyngeal and esophageal cancers confirmed

with upper gastrointestinal endoscopy.

Here, to determine whether an association exists between the *CYP2E1* Rsa I polymorphism and the risk of gastric cancer in a Japanese population, we performed a large-scale study of gastric cancer and noncancer control cases, as well as an age- and sex-matched case-control study. Neither study showed an association between *CYP2E1* Rsa I polymorphism and the risk of gastric cancer in the unoperated stomach. In this regard, a previous paper analyzing *CYP2E1* Rsa I polymorphism and gastric cancer risk in 2 case-control studies in Brazilians with or without Japanese ancestry¹⁸ reported that the Rsa I variant genotypes (C2/C2 or C1/C2) were less frequent in non-Japanese Brazilians with gastric cancer (OR=0.46) than in Japanese-Brazilians (OR=0.98). This frequency in Japanese-Brazilians is similar to that in our present Japanese population (OR=1.0). Even the presence of *H. pylori* infection cannot explain all causes of stomach carcinogenesis; in our study, the OR for *H. pylori* positivity as determined with an anti-IgG antibody and the risk of gastric cancer was 1.8 (95% CI=1.3-2.5). Our study also confirmed that serum pepsinogen I levels (≤ 50 ng/mL) and the pepsinogen I/pepsinogen II ratio (≤ 3.0) are good serum markers for predicting gastric cancer³⁰.

Conditions in the Stomach Cavity for the Activation of Carcinogenic Substances in the Unoperated and Remnant Stomach

The increased carcinogenic potential of gastric mucosa observed to have mucosal changes accompanying atrophic gastritis or intestinal metaplasia is thought to be due to 4 mechanisms, namely exposure to bile reflux, neutralization by the atrophy of acid-secreting chief cells, ongoing *H. pylori* infection³¹, and endogenous N-nitroso compounds³². These mechanisms also influence each other in gastric carcinogenesis. For example, a recent report has suggested that corpus-dominant gastritis shows a high risk for gastric carcinogenesis, especially in long-term *H. pylori* infection³³. Indeed, intestinal bacteria of the genus *Helicobacter* that grow in neutralized pH conditions have nitroso-reductase functions³⁴.

Important factors in remnant-stump carcinogenesis after distal gastrectomy include not only bile and intestinal juice reflux but also atrophic mucosal changes induced by long-term *H. pylori* infection⁸, especially in procedures involving Billroth II reconstruction³⁴. Bile juice usually inhibits the growth of *H. pylori*, but some *Helicobacter* species, such as *Helicobacter bilis*, can survive in bile and possess the enzyme activity required for nitroso reduction³⁵. Bile juice also contains other types of intestinal bacteria that can induce N-nitroso compounds in vivo^{24,32}, and some carcinogens are activated by liver enzymes. We have previously reported³⁶ that average pH levels in the unoperated stomach in *H. pylori*-positive patients were higher than those in *H. pylori*-negative patients and that neutralization in the stomach cavity induces the growth of certain intestinal bacteria that produce carcinogenic nitroso compounds in the stomach²². Normalization of pH level was also observed in the remnant stomach after the eradication of *H. pylori*³⁷. Here, these mucosal background factors were reflected in the positive association between this polymorphism and remnant stomach carcinogenesis, as opposed to those in the unoperated stomach.

In conclusion, we have identified a genotype-phenotype relationship between *CYP2E1* enzyme expression in the gastric mucosa. Possibly owing to our limited number of samples, however, no association was found between specific genotypes of the *CYP2E1* Rsa I polymorphism and this cancer risk. However, we speculate that individual variations in enzyme activity resulting from *CYP2E1* genetic polymorphisms might be associated with gastric carcinogenesis in the remnant stomach in particular conditions, such as with the neutralization of gastric pH levels by atrophic gastritis, intestinal metaplasia due to corpus-dominant gastritis by chronic *H. pylori* infection, or bile reflux after gastrectomy.

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