Original article

***Helicobacter pylori* infection, but not genetic polymorphism of CYP2E1, is highly prevalent in gastric cancer patients younger than 40 years**

GOTARO MASUDA¹, AKIRA TOKUNAGA², TAKASHI SHIRAKAWA³, AKIYOSHI TOGASHI¹, TERUO KIYAMA¹, SHUNJI KATO¹, NORIO MATSUKURA¹, HIDEKI BOU², MASANORI WATANABE², and TAKASHI TAJIRI¹

¹Surgery for Organ and Biological Regulation (First Department of Surgery), Nippon Medical School, Tokyo, Japan

²Institute of Gastroenterology, Nippon Medical School Musashikosugi Hospital, 1-396 Kosugicho, Nakahara-ku, Kawasaki, Kanagawa 211-8533, Japan

³Department of Surgery, Nippon Medical School Chiba Hokusoh Hospital, Chiba, Japan

Abstract

Background. Gastric cancers in young adults are thought to be associated with risk factors that include *Helicobacter pylori* infection and genetic polymorphism. The objective of this study was to elucidate the roles of these risk factors in patients younger than 40 years by analyzing clinicopathological data and *H. pylori* infection, and using molecular epidemiologic techniques.

Methods. Clinicopathological features, the presence of *H. pylori* infection, endoscopic characteristics of gastritis, genetic polymorphism of P4502E1 (CYP2E1), and family history of cancer in patients with gastric cancer treated surgically at Nippon Medical School Hospital from 1991 to 2004 were analyzed, based on our medical database.

Results. Gastric cancer in those younger than 40 years was characterized by a predominance of female patients with poorly differentiated adenocarcinoma who had undergone total gastrectomy with extended lymphadenectomy. *H. pylori* infection had a higher prevalence in patients with gastric cancer than in patients with normal endoscopic results or chronic gastritis, especially in those younger than 40 years (odds ratio, 13.7). Atrophic gastritis, nodular gastritis, and rugal hyperplastic gastritis were observed by endoscopy as *H. pylori*-associated gastritis. No difference in the incidence of either CYP2E1 genetic polymorphism or a family history of cancer was observed among different age groups.

Conclusion. Gastric cancer in patients younger than 40 years is closely associated with *H. pylori* infection, but not with genetic characteristics. Eradication therapy for *H. pylori* and endoscopic examination of *H. pylori*-positive young adults may be anticipated to be adopted as a strategy for the prevention and/or early detection of cancer.

Key words Gastric cancer · Young adults · *Helicobacter pylori*

Introduction

Genetic factors and/or exposure to environmental factors may contribute to the occurrence of human cancers. Risk factors for gastric cancer have been shown to include polymorphisms of nitroso compound activating enzyme [1,2], and the genetic polymorphisms [3–5] may be responsible for susceptibility to cancer as endogenous and/or host factors; as well, *Helicobacter pylori* infection [6–10], excessive sodium chloride intake [11], and Epstein-Barr (EB) virus infection [12] appear to play pivotal roles in the development of the cancer, as exogenous factors.

Reports [13–15] describing the occurrence of gastric cancer as being derived from the pathway of *H. pylori*-associated atrophic gastritis have been published. Long-term infection with *H. pylori* has been reported to result in *H. pylori*-associated chronic atrophic gastritis, which has been shown to correlate closely with gastric cancer, especially in young adults [16–19]. The endoscopic characteristics of *H. pylori*-associated gastritis in young adults have revealed nodular, rugal hyperplastic, and chronic atrophic gastritis to be specific to patients with *H. pylori* infection [20].

Cytochrome P4502E1 (CYP2E1) activates carcinogenic N-nitrosamines and other potential human carcinogens. Genetic polymorphisms of CYP2E1 have been shown to confer individual susceptibility to human cancers. Esophago-gastrointestinal cancers have been reported to be relevant to the expression of CYP2E1 and to N-nitrosamine metabolism [21–23]. It would thus be worthwhile to investigate whether the frequency of CYP2E1 restriction fragment-length polymorphism (RFLP) differs among gastric cancer patients younger than 40 years, patients in their sixties, and those 80 years or older.

The present studies were therefore designed to clarify the risk factors for gastric cancer in young adults;

the clinicopathological features of gastric cancer, based on clinical and pathological records; the incidences of *H. pylori* infection and *H. pylori*-associated gastritis, detected by blood tests and endoscopic biopsy; the frequency of CYP2E1 genetic polymorphism; and family history. Based on the results, current trials aimed at the reduction of risk factors are briefly discussed.

Patients and methods

A medical database containing retrospective documented variables in patients treated consecutively for gastric cancer at our hospital from 1991 to 2004 was analyzed. All patients had undergone gastrectomy with D0, D1, D2 or D3 type lymphadenectomy.

Clinicopathological studies

Clinicopathological findings, classified according to the Japanese Gastric Cancer Association *Japanese classification of gastric carcinoma* [24] and obtained from operative records and pathology reports, were filed using the File Maker Pro system (FileMaker, Santa Clara, CA, USA). The gastric cancer cases were then divided into early and advanced types according to the depth of tumor invasion in the gastric wall.

Surgical techniques

Five faculty surgeons, all specialists in gastric surgery, performed the operations. The most appropriate surgical procedure (total, proximal, or distal gastrectomy) was selected based on location, tumor size, and the extent of nodal involvement. For stage I gastric cancer surgery, the degree of lymph node dissection was selected at the surgeon's discretion. D2 dissection was the standard method for stages II and III, and D3 dissection was selected for nonelderly patients with stage III gastric cancer.

Endoscopic diagnosis of gastric cancer and H. pylori-associated gastritis

Endoscopic diagnosis was performed with gastrointestinal (GI) fiberscopes by a specialist in GI endoscopy. Retrospectively, the final diagnosis of gastric cancer and *H. pylori*-associated gastritis was made independently, based on observation of the endoscopic films by two GI endoscopy specialists. Any diagnostic discrepancy was discussed until agreement was reached. Criteria for endoscopic diagnosis were based on the Sydney system [25], and nodular gastritis was diagnosed according to previous reports [26,27].

Enzyme-linked immunosorbent assay (ELISA) for serum IgG antibody to H. pylori in patients with gastric cancer, those with peptic ulcer, and those with chronic gastritis who were endoscopically normal

Blood samples were collected and stored at -80°C until the assay. Serum IgG antibody to *H. pylori* was assayed by ELISA (AMRAD Operation, Australia), and absorbance at 450nm was measured. The results were expressed quantitatively, with less than 30 U/ml regarded as a negative result.

Detection of H. pylori by endoscopic biopsy in patients with gastric cancer, those with peptic ulcer, and those with chronic gastritis who were endoscopically normal

Endoscopic biopsy specimens were collected from: (1) the greater curvature of the antrum; (2) the greater curvature of the upper corpus; and (3) the lesser curvature of the lower corpus, for the detection of *H. pylori*. Hematoxylin-and-eosin staining, improved toluidine blue staining, and *H. pylori*-specific-antibody immunostaining (DAKO, Glostrup, Denmark) were used for the histological detection of *H. pylori* organisms.

Restricted fragment-length polymorphism (RFLP) analysis of CYP2E1

DNA was isolated from peripheral blood buffy coats by phenol extraction methods, as previously described. The polymerase chain reaction (PCR) was used to amplify the transcription regulatory region of CYP2E1, which includes the restriction enzyme recognition site for *RsaI*. Genomic DNA (0.1 μg) was amplified with primers (5'-TTCATTCTGTCTTCTAACTGG and 5'-TTCATTCTGTCTTCTAACTCG) according to previously reported methods [28]. The PCR product (15 μl) was subjected to *RsaI* restriction enzyme analysis by electrophoresis in an agarose gel (2.2%). The presence of the restriction site yielded two fragments, of 360 and 52bp.

Family history

Family histories of the patients with gastric cancer were obtained by interviews conducted by attending doctors, who spoke to the patients themselves and/or their relatives; the presence of cancer in second-degree relatives was taken as a positive family history.

Statistical analysis

Statistical analysis was checked by the χ^2 test, and cumulative survival rates were calculated using the Kaplan-Meier method and Stat View software (SAS

Institute, USA); survival curves were tested using the Logrank method. A *P*-value of less than 0.05 was considered significant.

Informed consent

All patients were informed of their therapeutic options. No treatments were performed without informed consent.

Ethics

The study protocol conformed to the ethical guidelines established by the 1975 Declaration of Helsinki and was approved by the institutional review board of Nippon Medical School.

Results

Clinicopathological features of gastric cancer in patients younger than 40 years

Patients younger than 40 years were characterized by a prevalence of female patients, mid-stomach cancer location, and poorly differentiated adenocarcinoma histologically.

Incidence of *H. pylori* infection

The *H. pylori* infection results in 31 patients younger than 40 years were used in this study. The positive rate in this group was 71% in males, and 88% in females. The positive rates for *H. pylori* in patients with gastric cancer regardless of age (*n* = 362) ranged from 70% to 80%, and were unaffected by age. The positive rates in patients with normal endoscopic findings and chronic gastritis (*n* = 721) were 23.3% in those younger than 40 years, 48.4% in patients in their forties, and 56.8%,

61.7%, 60.7%, and 55.6% in patients in their fifties, sixties, seventies, and those 80 years or older, respectively. In the peptic ulcer group (*n* = 939), the positive rate was more than 70% in those in their thirties to sixties, but the prevalence declined after the seventies. The odds ratios for *H. pylori* infection in patients with gastric cancer and the patients with normal endoscopic findings and chronic gastritis are shown in Table 1, the highest odds ratio was 13.69, in patients younger than 40 years. Odds ratios were higher in patients with gastric cancer than in those with normal endoscopic findings and chronic gastritis, regardless of age.

H. pylori-associated gastritis

Fifteen of the 31 (48.4%) patients aged less than 40 years were diagnosed as having atrophic gastritis in the gastric body; 3 of the 31 (9.7%) also had nodular gastritis in the antrum, and 1 of the 31 (3.2%) showed rugal hyperplastic gastritis in the gastric body.

RFLP analysis of CYP2E1

The results of the CYP2E1 polymorphism analysis in patients with gastric cancer are shown in Table 2, stratified by age group for those younger than 40 years, those in their sixties, and those 80 years or older. In the CYP2E1 *RsaI* restriction analysis, the homozygous minor genotype (R/R) and heterozygous genotype (C/R) were not found to differ significantly among the three groups.

Family history of cancer

Table 2 also shows the prevalence of a family history of cancer, stratified by the above three age groups. No significant differences were observed among the three groups.

Table 1. Odds ratios for *Helicobacter pylori* infection positive rates in patients with gastric cancer versus patients with normal endoscopic findings and chronic gastritis

Age (years)	<i>H. pylori</i> infection		Odds ratio (95% CI)
	Gastric cancer	Endoscopically normal + chronic gastritis	
≤39	25/31 (80.6%)	28/120 (23.3%)	13.69 (5.11–36.71)
40–49	36/49 (73.5%)	46/95 (48.4%)	2.95 (1.39–6.25)
50–59	52/67 (77.6%)	104/183 (56.8%)	2.63 (1.38–5.02)
60–69	94/128 (73.4%)	111/180 (61.7%)	1.72 (1.05–2.82)
70–79	49/64 (76.6%)	65/107 (60.7%)	2.11 (1.05–4.24)
≥80	16/23 (69.6%)	20/36 (55.6%)	1.83 (0.61–5.52)

Table 2. Comparison of genetic characteristics of patients with gastric cancer among three groups (patients younger than 40 years, patients in their sixties, and patients 80 years or older), showing prevalence of cytochrome P4502E1 (CYP2E1) genetic polymorphism and prevalence of a family history of cancer

	Younger than 40 years	Sixties	80 years or older
Genetic polymorphism of CYP2E1			
C/C ^C	17/31 (54.8%)	137/231 (59.3%)	18/38 (47.4%)
C/R + R/R ^C	14/31 (45.2%)	94/231 (40.7%)	20/38 (52.6%)
	N.S.		
	N.S.		
Family history			
No	20/31 (64.5%)	246/342 (71.9%)	47/62 (75.8%)
Yes	11/31 (35.5%)	96/342 (28.1%)	15/62 (24.2%)
	N.S.		
	N.S.		

N.S., not significant

Discussion

In 31 patients younger than 40 years with gastric cancer who underwent surgery between 1991 and 2004, the clinicopathological features, presence of *H. pylori* infection, and characteristics of gastritis were examined; CYP2E1 genetic polymorphism and family history were also investigated, in comparison with findings in patients with gastric cancer in their sixties and 80 years or older.

Clinicopathological features in the 31 patients younger than 40 years with gastric cancer were characterized by the predominance of female patients with poorly differentiated adenocarcinoma, located in the middle part of the stomach, many of whom had undergone total gastrectomy with D2 or D3 lymph node dissection. The predominance of female patients with poorly differentiated adenocarcinoma that we observed was in accordance with other reports [29,30].

As compared with the results in the patients with normal endoscopic findings and chronic gastritis, the predominance of *H. pylori* infection in the patients younger than 40 was striking; 71% of the male and 88% of the female patients being positive for *H. pylori*. Gastric cancer in young adults has been found to be associated with *H. pylori* infection. An increasing number of reports have stressed the association of *H. pylori* infection with the development of gastric cancer in young adults [16–18]. The question of the role of *H. pylori* infection in the gastric mucosa in the development of the diffuse type of cancer without mucosal atrophy remains open, in contrast to the role of this infection in the intestinal type of cancer with mucosal atrophy and intestinal metaplasia. The relationship between *H.*

Fig. 1. *Helicobacter pylori* infection positive rates according to gastric disease for various age groups. Continuous line, patients with gastric cancer ($n = 362$); dashed line, patients with gastric ulcer + duodenal ulcer (peptic ulcer; $n = 939$); dotted/dashed line, patients with normal endoscopic findings + chronic gastritis ($n = 721$)

pylori-associated gastritis and the development of the diffuse type of cancer has been considered by Uemura et al. [14], who noted that gastric mucosa with severe inflammation and moderate atrophy induced by *H. pylori* infection had a potential risk for undifferentiated gastric cancer. Miyamoto et al. [26,27] have reported that nodular gastritis which is induced by *H. pylori* infection is closely associated with gastric cancer in young adults. A bird-skin appearance in endoscopy and hyperplasia of B-lymphocytes are characteristics of nodular gastritis, which is considered to represent marked inflammation. Active and chronic inflammatory cellular infiltrations were found to correlate with *H. pylori* in-

fection in a study of gastric remnants [31]. However, there is a limit to the relevance of discussing gastric cancer development in various kinds of surgically resected specimens. *H. pylori* infection evidently causes inflammation of the gastric mucosa, and cytokines derived from inflammatory cells may transform epithelial or mesenchymal cells, resulting in gastric cancer or mucosa-associated lymphoid tissue (MALT) lymphoma. A recent report has reviewed the clinical outcomes of gastric lesions induced by *H. pylori* infection, which depend mainly on host factors [32].

Other recent reports have suggested that CYP2E1 genetic polymorphism is responsible for susceptibility to GI cancers as well as lung cancer [21–23]. The present study yielded negative results regarding an association of CYP2E1 genetic polymorphism with the occurrence of gastric cancer in patients younger than 40 years. No association of CYP2E1 genetic polymorphism or other genetic factors with gastric cancer has been reported previously [28].

Familial clustering of cancer in patients with gastric cancer was studied in the present investigation, but no difference in the prevalence of family history was found among the three age groups we examined. Familial aggregation was reported to be specific for the intestinal type of gastric cancer in a patient with a positive family history [33]. Intrafamilial clustering of *H. pylori* infection was reported to have a crucial role in familial aggregation of gastric cancer [34].

In conclusion, a high prevalence of *H. pylori* infection in patients with gastric cancer younger than 40 years was confirmed in the present study. Eradication therapy for *H. pylori* would therefore be expected to prevent gastric cancer development, as this cancer arises from *H. pylori*-associated gastritis. Furthermore, endoscopic GI examinations in people who are *H. pylori*-positive would promote the early detection of cancer. Trials of *H. pylori* eradication therapy for the prevention of gastric cancer are currently underway in China and Japan, and promising results are being obtained [35,36].

References

1. Yang CS, Yoo JS, Ishizaki H, Hong JY. Cytochrome P450IIE1: role in nitrosamine metabolism and mechanisms of regulation. *Drug Metab Rev* 1990;22:147–9.
2. Guengerich FP, Kim DH, Iwasaki M. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem Res Toxicol* 1991;4:167–79.
3. Bevan S, Houlston RS. Genetic predisposition to gastric cancer. *QJM* 1999;92:5–10.
4. Miao X, Xing D, Tan W, Qi J, Lu W, Lin D. Susceptibility to gastric cardia adenocarcinoma and genetic polymorphism in methylenetetrahydrofolate reductase in an at-risk Chinese population. *Cancer Epidemiol Biomarkers Prev* 2002;11:1454–8.
5. Zhu Y, Spitz MR, Amos CI, Lin MB, Schabath MB, Wu X. An evolutionary perspective on single-nucleotide polymorphism screening in molecular cancer epidemiology. *Cancer Res* 2004;64:2251–7.
6. Blaser MJ, Perez-Perez GI, Kleanthous H, Cover TL, Peek RM, Chyou PH, et al. Infection with *Helicobacter pylori* strains possessing cagA is associated with an increased risk of developing adenocarcinoma of the stomach. *Cancer Res* 1995;55:2111–5.
7. Uemura N, Mukai T, Okamoto S, Yamaguchi S, Mashiba H, Taniyama K, et al. Effect of *Helicobacter pylori* eradication on subsequent development of cancer after endoscopic resection of early gastric cancer. *Cancer Epidemiol Biomarkers Prev* 1997;6:639–42.
8. Parsonnet J, Friedman GD, Orentreich N, Vogelstein H. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. *Gut* 1997;40:297–301.
9. Kikuchi S. Epidemiology of *Helicobacter pylori* and gastric cancer. *Gastric Cancer* 2002;5:6–15.
10. El-Omar EM, Rabkin CS, Gammon MD, Vaughan TL, Risch HA, Schoenberg JB, et al. Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003;124:1193–201.
11. Shikata K, Kiyohara Y, Kubo M, Yonemoto K, Ninomiya T, Shiota T, et al. A prospective study of dietary salt intake and gastric cancer incidence in a defined Japanese population: The Hisayama study. *Int J Cancer* 2006;119:196–201.
12. Burke AP, Yen TS, Shekitka KM, Sobin LH. Lymphoepithelial carcinoma of the stomach with Epstein-Barr virus demonstrated by polymerase chain reaction. *Mod Pathol* 1990;3:377–80.
13. Correa P. Human gastric carcinogenesis: a multistep and multifactorial process.—First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;52:6735–40.
14. Uemura N, Okamoto S, Yamamoto S, Matsumura N, Yamaguchi S, Yamakido M, et al. *Helicobacter pylori* infection and the development of gastric cancer. *N Engl J Med* 2001;345:784–9.
15. Takahashi S. Long-term *Helicobacter pylori* infection and the development of atrophic gastritis and gastric cancer in Japan. *J Gastroenterol* 2002;37:24–7.
16. Kikuchi S, Wada O, Nakajima T, Nishi T, Kobayashi O, Konishi T, et al. Serum anti-*Helicobacter pylori* antibody and gastric carcinoma among young adults. *Cancer* 1995;75:2789–93.
17. Kokkola A, Valle J, Haapiainen R, Sipponen P, Kivilaakso E, Puolakkainen P. *Helicobacter pylori* infection in young patients with gastric carcinoma. *Scand J Gastroenterol* 1996;31:643–7.
18. Koshida Y, Koizumi W, Sasabe M, Katoh Y, Okayasu I. Association of *Helicobacter pylori*-dependent gastritis with gastric carcinomas in young Japanese patients: histopathological comparison of diffuse and intestinal type cancer cases. *Histopathology* 2000;37:124–30.
19. Riquarte O, Gutierrez O, Cardona H, Kim JG, Graham DY, El-Zimaity HM. Atrophic gastritis in young children and adolescents. *Clin Pathol* 2005;58:1189–93.
20. Shimatani T, Inoue M, Iwamoto K, Hyogo H, Yokozaki M, Saeki T, et al. Prevalence of *Helicobacter pylori* infection, endoscopic gastric findings and dyspeptic symptoms among a young Japanese population born in the 1970s. *J Gastroenterol Hepatol* 2005;20:1352–7.
21. Godoy W, Albano RM, Moraes EG, Alves Pinho PR, Nues RA, Saito EH, et al. CYP2A6/2A7 and CYP2E1 expression in human oesophageal mucosa; regional and inter-individual variation in expression and relevance to nitrosamine metabolism. *Carcinogenesis* 2002;23:611–6.
22. Marchand LL, Donlon T, Seifried A, Wilkens LR. Red meat intake, CYP2E1 polymorphisms, and colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 2002;11:1019–24.
23. Cai L, Zheng ZL, Zheng ZF. Cytochrome p450 2E1 polymorphisms and the risk of gastric cardia cancer. *World J Gastroenterol* 2005;11:1867–71.

24. Japanese Gastric Cancer Association. Japanese classification of gastric carcinoma. 2nd English Ed. Gastric Cancer 1998;1:10–24.
25. Tytgat GN. The Sydney System: endoscopic division. Endoscopic appearances in gastritis/duodenitis. J Gastroenterol Hepatol 1991; 6:223–34.
26. Miyamoto M, Haruma K, Yoshihara M, Hiyama T, Sumioka M, Nishisaka T, et al. Nodular gastritis in adults is caused by *Helicobacter pylori* infection. Dig Dis Sci 2003;48:968–75.
27. Miyamoto M, Haruma K, Yoshihara M, Sumioka M, Nishisaka T, Tanaka S, et al. Five cases of nodular gastritis and gastric cancer: a possible association between nodular gastritis and gastric cancer. Dig Liver Dis 2002;34:819–20.
28. Kato S, Onda M, Matsukura N, Tokunaga A, Tjiri T, Kim DY et al. Cytochrome P450 (CYP2E1) genetic polymorphism in a case-control study of gastric cancer and liver disease. Pharmacogenetics 1995;5:s141–4.
29. Ramos-De la Medina A, Salgado-Nesme N, Torres-Villalobos G, Medina-Franco H. Clinicopathologic characteristics of gastric cancer in a young patient population. J Gastroint Surg 2004;8: 240–4.
30. Llanos O, Butte JM, Crovari F, Duarte I, Guzman S. Survival of young patients after gastrectomy for gastric cancer. World J Surg 2006;30:17–20.
31. Lee Y, Tokunaga A, Tajiri T, Masuda G, Okuda T, Fujita I, et al. Inflammation of the gastric remnant after gastrectomy: mucosal erythema is associated with bile reflux and inflammatory cellular infiltration is associated with *Helicobacter pylori* infection. J Gastroenterol 2004;39:520–6.
32. Chiba T, Seno H, Marusawa H, Wakatsuki Y, Okazaki K. Host factors are important in determining clinical outcome of *Helicobacter pylori* infection. J Gastroenterol 2006;41:1–9.
33. Bernini M, Barbi S, Roviello F, Scarpa A, Moore P, Pedrazzani C, et al. Family history of gastric cancer: a correlation between epidemiologic findings and clinical data. Gastric Cancer 2006;9: 9–13.
34. Chang YW, Han YS, Lee DK, Kim HJ, Moon JS, Dong SH, et al. Role of *Helicobacter pylori* infection among offspring or siblings of gastric cancer patients. Int J Cancer 2002;101:469–74.
35. Wong BC, Lam SK, Wong WM, Chen JS, Zheng TT, Feng RE, et al. *Helicobacter pylori* eradication to prevent gastric cancer in a high risk region of China: a randomized controlled trial. JAMA 2004;291:344–6.
36. Saito D. *H. pylori* and gastric cancer: Japanese Intervention Trial of *H. pylori*. In: Proc 78th Japan Gastric Cancer Assoc; 2006. p. 163.