

## Impact of Catechol-*O*-methyltransferase (*COMT*) Gene Polymorphism on Promoter Methylation Status in Gastric Mucosa

TOMOMITSU TAHARA, TOMOYUKI SHIBATA, TOMIYASU ARISAWA, MASAKATSU NAKAMURA,  
HIROMI YAMASHITA, DAISUKE YOSHIOKA, MASAAKI OKUBO, NAOKO MARUYAMA,  
TOSHIAKI KAMANO, YOSHIO KAMIYA, HIROSHI FUJITA, MITSUO NAGASAKA,  
MASAMI IWATA, KAZUYA TAKAHAMA, MAKOTO WATANABE and ICHIRO HIRATA

*Department of Gastroenterology, Fujita Health University School of Medicine,  
Kutsukake-cho, Toyoake, Aichi, 470-1192, Japan*

**Abstract.** DNA methylation is one of the major events in the early process of gastric carcinogenesis and also occurs in non-neoplastic gastric mucosa. Catechol-*O*-methyltransferase (*COMT*) catalyzes the methylation of various endobiotic and xenobiotic substances, and protects DNA from oxidative damage. The association between a common functional polymorphism of *COMT* Val158Met and DNA methylation status in the stomach was investigated. **Patients and Methods:** One hundred and sixty-nine gastric mucosa samples from non-cancer patients were obtained by endoscopy. The promoter methylation status of *p14* and *p16* was determined by methylation-specific PCR (MSP). The *COMT* Val158Met polymorphism was detected by PCR-restriction fragment length polymorphism (RFLP). **Results:** CpG island methylation was observed in 32.5% of the *p14*, and 37.9% of the *p16*. The methylation status of both *p14* and *p16* was not associated with gender or age, while *p16* methylation was strongly associated with *Helicobacter pylori* infection ( $OR=4.71$ , 95% CI=2.35-9.46,  $p<0.0001$ ). The Val/Val genotype held a significantly higher risk of *p16* methylation ( $OR=3.27$ , 95% CI=1.05-10.25,  $p=0.0418$ ). **Conclusion:** The *COMT* polymorphism may influence the susceptibility to gene methylation in the

gastric mucosa. The promoter CpG island of *p16* gene, but not of *p14* may be one of the specific regions whose methylation is closely influenced by the *COMT* polymorphism.

Aberrant DNA methylation is an important mechanism in gene silencing. In many kinds of cancer, some genes seem to acquire aberrant methylation in their CpG islands. Some genes are also methylated in non-neoplastic tissues with aging and this alteration is known as age-related methylation (1, 2). In addition, it has been shown that gene methylation may be present in non-neoplastic colorectal mucosa in patients with inflammatory bowel disease (3, 4), esophageal mucosa in patients with Barrett's esophagitis (5, 6) and liver tissues in chronic hepatitis (7).

In the non-neoplastic human gastric mucosa, frequencies or levels of CpG islands methylation of certain genes correlate with *Helicobacter pylori* infection (8, 9), histological or serological severity of gastritis and gastric cancer occurrence (10-13), suggesting that aberrant DNA methylation is one of the major events which occurs early in the process of tumorigenesis in the stomach. These epigenetic events in the gastric mucosa may lead to transcriptional inactivation in specific genes and increase DNA damage or mutation and chromosomal instability.

*p16(INK4a)* and *p14(ARF)* are involved in the negative cell cycle regulation via the pRb and p53 pathways, respectively. The genes for these two proteins have an independent first exon (exon 1a and 1 $\beta$ , respectively) but share exons 2 and 3 (14, 15). Methylation of *p16* and *p14* has been shown to be present in gastric cancer as well as premalignant lesions (16, 17). Thus, both genes may play crucial roles in cell cycle control, apoptosis and DNA repair in the stomach and their disorder may be closely associated with gastric carcinogenesis.

**Abbreviations:** COMT, Catechol-*O*-methyltransferase; MSP, methylation-specific polymerase chain reaction.

**Correspondence to:** Tomomitsu Tahara, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake, Aichi, 470-1192, Japan Tel: +81 562939240, Fax: +81 562938300, e-mail: tomomiccyu@yahoo.co.jp

**Key Words:** Methylation, *Helicobacter pylori*, gastric mucosa, *COMT*, polymorphism.

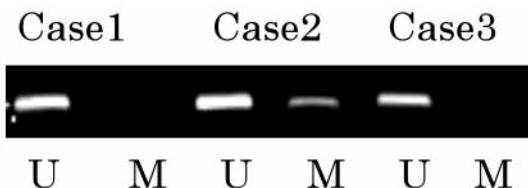


Figure 1. Hypermethylation of promoter region of *p14* in gastric mucosa in 3 cases. DNA from specimens of gastric mucosa in case 2 showed a positive methylated band. U; un-methylated band M; methylated band.

Catechol-*O*-methyltransferase (COMT) is expressed in various mammalian tissues including the stomach (18-20) and has been shown to catalyze the methylation of various endobiotic and xenobiotic substances preventing quinone formation and redox cycling, and therefore might protect DNA from oxidative damage (21, 22). A G to A transition, which results in an amino acid change from valine to methionine at codon 158, leads to COMT activity of the Met/Met genotype which is a quarter of that of the wild genotype, and heterozygous individuals exhibit intermediate enzyme activity (23).

Because of the important role that COMT plays with respect to preventing DNA damage, the polymorphism of *COMT* may influence the susceptibility to methylation in the human gastric mucosa.

In the present study, the methylation status of *p14* and *p16* in non-neoplastic gastric mucosa samples and its relation to the *COMT* Val158Met polymorphism was investigated.

## Patients and Methods

**Tissue samples and DNA extraction.** The study population comprised 169 non-cancer patients, attending the Endoscopy Center of Fujita Health University Hospital from January 2005 to July 2007. All patients underwent upper gastroscopy as part of a health check, as a secondary procedure following barium X-ray examination for suspected stomach cancer, or for the investigation of abdominal discomfort. Patients who had severe systemic disease, or malignancy in the stomach or other organ were excluded from this study. Biopsy specimens were taken from the antrum along the greater curvature, from grossly non-pathological mucosa in all patients. The specimens were cut into two pieces. One of the pieces was fixed in 10% buffered formalin and embedded in paraffin for microscopic histological examination and the other part was immediately frozen and stored at -80°C until use. Histological analysis of all the selected biopsy samples also showed that these samples contained more than 70% of epithelial cells. Genomic DNA was extracted directly from the frozen specimens using a standard phenol/chloroform method. *H. pylori* infection status was assessed by serological, histological analysis or urea breath test. Patients were diagnosed as infected when at least one of the diagnostic tests was positive. The Ethics Committee of the Fujita Health University School of Medicine approved the protocol and prior, written informed consent was obtained from all participants.

Table I. Characteristics of the 169 patients.

Variable	
Age (mean±SD)	60.0±13.1
Gender (male/female)	94/75
<i>H. pylori</i> infection (+/-)	77/92
Active ulcer disease	13

**Bisulfite modification and methylation-specific PCR (MSP).** For the examination of DNA methylation, the genomic DNA was treated with sodium bisulfite using a Bisulfite DNA Modification Kit for Methylated DNA Detection (Toyobo, Co. Ltd., Osaka, Japan). MSP was carried out with the following primers: *p14* methylated forward (MF), 5'-gtgttaaaggcgccgtacg-3' and *p14* methylated reverse (MR), 5'-aaaaccctactcgacga-3' which amplify a 122-bp product; *p14* unmethylated forward (UF), 5'-ttttgggtttaaagggttgtgt-3' and *p14* unmethylated reverse (UR), 5'-cacaaaaaccctactacaacaa-3' which amplify a 132-bp product (24); *p16* methylated forward (MF), 5'-tttagagggtggcgatgc-3' and *p16* methylated reverse (MR), 5'-accccgaaaccgcgaccgtaa-3' which amplify a 149-bp product; *p16* unmethylated forward (UF), 5'-tttagagggtgggtggattgt-3' and *p16* unmethylated reverse (UR), 5'-caaccccaaaccacaaccataa-3' which amplify a 151-bp product (25). The annealing temperature and times were determined using DNA from the peripheral blood of a young individual without *H. pylori* infection and DNA methylated with *SssI* methylase (New England BioLabs Inc., Beverly, MA, USA). The MSP was carried out in a volume of 21 µL containing 0.1 µg of bisulfite-modified DNA. The DNA was denatured at 95°C for 5 minutes, followed by 33–35 cycles at 95°C for 30 s and according to the primers for 1 minute, and 72°C for 1 minute with a final extension at 72°C for 5 minutes. The MSP reactions were conducted using EX Taq HS (Takara Bio Inc., Shiga, Japan). The bands of MSP were detected by electrophoresis in 2.5% agarose gels stained with ethidium bromide (Figure 1).

**COMT Val158Met genotypes.** Using genomic DNA, the polymorphism at codon 158 in the *COMT* gene was determined by PCR-restriction fragment length polymorphism (RFLP) assays. The PCR primers 5'-tcgtggacccgtgttcagg-3' and 5'-aggtctgacaacgggtcaggc-3' were used to amplify a 217-bp fragment of *COMT* that contains the polymorphic *Nla*III site as well as one other constant *Nla*III site. PCR was performed in a reaction volume of 25 µL containing 200 ng of genomic DNA, 10 pmol of each primer, 200 ng of each dNTP and 0.6 units Taq DNA polymerase (Toyobo). The reaction mixture was first denatured at 95°C for 5 min and then amplified by PCR for 32 cycles at 95°C for 30 s, at 55°C for 30 s, and at 72°C for 1 min, followed by a 10 min extension at 72°C. Twelve µL of PCR product were incubated with 10 units of *Nla*III (New England Biolabs) in a volume of 20 µL at 37°C overnight. *Nla*III cuts only the low-activity variant (*COMT*158Met) in addition to a second constant cleavage site in the PCR product. Thus, homozygotes for *COMT*158Val generated fragments of 136 and 81 bp, heterozygotes gave 136, 96, 81, and 40 bp fragments and homozygotes for *COMT*158Met generated 96, 81, and 40-bp fragments. The 40bp fragment ran off the gel during electrophoresis. The 3 genotypes were scored after running on a 3.5% agarose gel with ethidium bromide 10 µg/mL.

Table II. Association between p14 and p16 promoter methylation and age, gender, *H. pylori* infection and COMT Val158Met genotypes.

Variable (n)	Age (Mean±SD) (years)	Gender (n)		<i>H. pylori</i> infection (n) <sup>a</sup>		COMT genotype (n) <sup>b</sup>		
		Male (94)	Female (75)	- (77)	+(92)	Val/Val (80)	Val/Met (75)	Met/Met (14)
<i>p14</i>								
Unmethylated (114)	58.5±13.3	66	48	54	60	56	47	11
Methylated (55)	63.2±12.0	28	27	23	32	24	28	3
<i>p16</i>								
Unmethylated (105)	60.5±14.0	59	46	62	43	49	51	5
Methylated (64)	59.4±11.5	35	29	15	49	31	24	9
<i>p14 or p16</i>								
Unmethylated (69)	58.3±14.5	42	27	42	27	35	30	4
Either methylated (100)	61.2±11.9	52	48	35	65	45	45	10
Both <i>p14</i> and <i>p16</i>								
Unmethylated (150)	60.0±13.3	83	67	74	76	70	68	12
Both methylated (19)	60.6±11.6	11	8	3	16	10	7	2

<sup>a</sup>*p16*, + vs. -: OR=4.71, 95% CI=2.35-9.46, *p*<0.0001; *p14 or p16*, + vs. -: OR=2.89, 95% CI=1.53-5.45, *p*=0.001; Both *p14* and *p16*, + vs. -: OR=5.19, 95% CI=1.45-18.57, *p*=0.006; <sup>b</sup>*p16*, Met/met vs. Val/Val+Val/Met, OR=3.27, 95% CI=1.05-10.25, *p*=0.0418.

**Statistical analysis.** Statistical analysis was conducted with two-sided chi-square for the comparison of promoter DNA methylation frequencies of *p14* and *p16* between two groups. The association between DNA methylation status of *p14* and *p16* and age was examined by the Mann-Whitney *U*-test. A probability value of less than 0.05 was considered statistical significant.

## Results

**Study population.** The characteristics of the participants is shown in Table I. After gastroscopy, 13 patients (7.7%) were diagnosed as having active peptic ulcer disease.

**Association between methylation of *p14* and *p16* and gender, age, *H. pylori* infection and COMT Val158Met polymorphism.** All 169 gastric mucosa samples were available for MSP analysis and COMT genotyping. CpG island methylation of *p14* was observed in 55 (32.5%) and of *p16* in 64 subjects (37.9%). The methylation status of both *p14* and *p16* was not associated with gender or age, while *p16* methylation was strongly associated with *H. pylori* infection (OR=4.71, 95% CI=2.35-9.46, *p*<0.0001). The COMT genotype distribution in the 169 participants was 80 Val/Val (47.3%), 75 Val/Met (44.4%) and 14 Met/Met (8.3%). Although no association was found between the COMT genotypes and *p14* methylation, the Met/Met genotype was significantly associated with *p16* methylation (Met/Met vs. Val/Val/ + Val/Met; OR=3.27, 95% CI=1.05-10.25, *p*=0.0418). While such an association was not found for the Met carriers (Val/Val vs. Met carriers; OR= 0.93, 95% CI=0.50-1.74, *p*=0.82). The prevalence of patients in whom either *p14* or *p16* or both *p14* and *p16* were methylated was also investigated. Although *H. pylori* infection was significantly associated with

methylation of either, and both *p14* and *p16* (*p14 or p16*: OR=2.89, 95% CI=1.53-5.45, *p*=0.001, both *p14* and *p16*: OR=5.19, 95% CI=1.45-18.57, *p*=0.006), age, gender and COMT genotype were not associated with methylation of either *p14* or *p16* or both *p14* and *p16* (Table II).

## Discussion

Methylation of *p16* was strongly associated with *H. pylori* infection. This observation is in line with previous studies (8-13). In regard to the CpG sites, the Met/Met genotype held a significantly higher risk of *p16* rather than *p14* promoter methylation.

The amino acid change at codon 158 (Val to Met) of COMT results in lower thermostability, with enzyme activity that is up to four times less than that from the wild-type allele (26). The presence of the Val/Val genotype has been considered to be favorable because it seems to lower the risk of developing non-Hodgkin lymphoma and estrogen-associated carcinomas in women (27-29), and because it is associated with a higher tendency to remain free from an increase in prostate-specific antigen in men with prostate cancer (30).

The mechanisms of gene methylation are unknown. Several factors may contribute to this methylation, such as exogenous carcinogens, generated reactive oxygen, and host genetic differences (31). One of the most important factors causing oxidative stress in the gastric mucosa is *H. pylori* infection, which induces chronic inflammation (32, 33). Indeed, the methylation of certain genes in non-neoplastic gastric mucosa correlates with *H. pylori* infection (8, 9), histological or serological severity of gastritis and

gastric cancer occurrence (11-13). However, not all patients with *H. pylori* infection or gene methylation develop gastric cancer. This difference may be attributed to some genetic factors. The present results provided the first evidence that the genetic polymorphisms of *COMT* may also be involved in DNA methylation in human gastric mucosa. Although the activity of COMT in the serum or gastric mucosa was not investigated, it is possible that the *COMT* polymorphism influences the activity and modifies the risk of DNA methylation in the gastric mucosa, and thus, influences the susceptibility to methylation-related carcinogenesis.

Meanwhile, no significant association between *COMT* polymorphism and *p14* methylation status was found. In addition, the *COMT* genotype was also not associated with methylation of either *p14* or *p16*. The promoter CpG island of *p16* gene, but not of *p14* may be one of the specific regions whose methylation is closely influenced by *COMT* polymorphism. However, why the interplay between *COMT* polymorphism and aberrant hypermethylation is different in different genes is still unexplained. Only a more extensive understanding of the regulation of methylation in relation to gene expression and carcinogenesis will allow us to fully interpret our findings.

## References

- 1 Issa JP, Ottaviano YL, Celano P, Hamilton SR, Davidson NE and Baylin SB: Methylation of the oestrogen receptor CpG island links ageing and neoplasia in human colon. *Nat Genet* 7: 536-540, 1994.
- 2 Ahuja N, Li Q, Mohan AL, Baylin SB and Issa JP: Aging and DNA methylation in colorectal mucosa and cancer. *Cancer Res* 58: 5489-5494, 1998.
- 3 Sato F, Harpaz N, Shibata D, Xu Y, Yin J, Mori Y, Zou TT, Wang S, Desai K, Leytin A, Selaru FM, Abraham JM and Meltzer SJ: Hypermethylation of the *p14(ARF)* gene in ulcerative colitis-associated colorectal carcinogenesis. *Cancer Res* 62: 1148-1151, 2002.
- 4 Issa JP, Ahuja N, Toyota M, Bronner MP and Brentnall TA: Accelerated age-related CpG island methylation in ulcerative colitis. *Cancer Res* 61: 3573-3577, 2001.
- 5 Bian YS, Osterheld MC, Fontolliet C, Bosman FT and Benhantar J: *p16* inactivation by methylation of the *CDKN2A* promoter occurs early during neoplastic progression in Barrett's esophagus. *Gastroenterology* 122: 1113-1121, 2002.
- 6 Wong DJ, Paulson TG, Prevo LJ, Galipeau PC, Longton G, Blount PL and Reid BJ: *p16(INK4a)* lesions are common, early abnormalities that undergo clonal expansion in Barrett's metaplastic epithelium. *Cancer Res* 61: 8284-8289, 2001.
- 7 Kaneto H, Sasaki S, Yamamoto H, Itoh F, Toyota M, Suzuki H, Ozeki I, Iwata N, Ohmura T, Satoh T, Karino Y, Satoh T, Toyota J, Satoh M, Endo T, Omata M and Imai K: Detection of hypermethylation of the *p16(INK4A)* gene promoter in chronic hepatitis and cirrhosis associated with hepatitis B or C virus. *Gut* 48: 372-377, 2001.
- 8 Chan AO, Lam SK, Wong BC, Wong WM, Yuen MF, Yeung YH, Hui WM, Rashid A and Kwong YL: Promoter methylation of *E-cadherin* gene in gastric mucosa associated with *Helicobacter pylori* infection and in gastric cancer. *Gut* 52: 502-506, 2003.
- 9 Maekita T, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M, Arii K, Kaneda A, Tsukamoto T, Tatematsu M, Tamura G, Saito D, Sugimura T, Ichinose M and Ushijima T: High levels of aberrant DNA methylation in *Helicobacter pylori*-infected gastric mucosae and its possible association with gastric cancer risk. *Clin Cancer Res* 12: 989-995, 2006.
- 10 Kang GH, Lee HJ, Hwang KS, Lee S, Kim JH and Kim J: Aberrant CpG island hypermethylation of chronic gastritis, in relation to aging, gender, intestinal metaplasia, and chronic inflammation. *Am J Pathol* 163: 1551-1556, 2003.
- 11 Tahara T, Arisawa T, Shibata T, Wang FY, Nakamura M, Sakata M, Nagasaka M, Takagi T, Kamiya Y, Fujita H, Nakamura M, Hasegawa S, Iwata M, Takahama K, Watanabe M, Hirata I and Nakano H: Risk prediction of gastric cancer by analysis of aberrant DNA methylation in non-neoplastic gastric epithelium. *Digestion* 75: 54-61, 2007.
- 12 Kaise M, Yamasaki T, Yonezawa J, Miwa J, Ohta Y and Tajiri H: CpG island hypermethylation of tumor-suppressor genes in *H. pylori*-infected non-neoplastic gastric mucosa is linked with gastric cancer risk. *Helicobacter* 13: 35-41, 2008.
- 13 Nakajima T, Maekita T, Oda I, Gotoda T, Yamamoto S, Umemura S, Ichinose M, Sugimura T, Ushijima T and Saito D: Higher methylation levels in gastric mucosae significantly correlate with higher risk of gastric cancers. *Cancer Epidemiol Biomarkers Prev* 15: 2317-2321, 2006.
- 14 Rizos H, Darmanian AP, Mann GJ and Kefford RF: Two arginine rich domains in the *p14<sup>ARF</sup>* tumour suppressor mediate nucleolar localization. *Oncogene* 19: 2978-2985, 2000.
- 15 Tannapfel A, Busse C, Weinans L, Benicke M, Katalinic A, Geissler F, Hauss J and Wittekind C: *INK4a-ARF* alterations and *p53* mutations in hepatocellular carcinomas. *Oncogene* 20: 7104-7109, 2001.
- 16 Toyota M, Ahuja N, Suzuki H, Itoh F, Ohe-Toyota M, Imai K, Baylin SB and Issa JP: Aberrant methylation in gastric cancer associated with the CpG island methylator phenotype. *Cancer Res* 59: 5438-5442, 1999.
- 17 Kang GH, Shim YH, Jung HY, Kim WH, Ro JY and Rhyu MG: CpG island methylation in premalignant stages of gastric carcinoma. *Cancer Res* 61: 2847-2851, 2001.
- 18 Matsumoto M, Weickert CS, Beltaifa S, Kolachana B, Chen J, Hyde TM, Herman MM, Weinberger DR and Kleinman JE: Catechol *O*-methyltransferase (*COMT*) mRNA expression in the dorsolateral prefrontal cortex of patients with schizophrenia. *Neuropsychopharmacology* 28: 1521-1530, 2003.
- 19 Tenhunen J, Salminen M, Jalanko A, Ukkonen S and Ulmanen I: Structure of the rat *catechol-O-methyltransferase* gene: separate promoters are used to produce mRNAs for soluble and membrane-bound forms of the enzyme. *DNA Cell Biol* 12: 253-263, 1993.
- 20 Lundstrom K, Tenhunen J, Tilgmann C, Karhunen T, Panula P and Ulmanen I: Cloning, expression and structure of catechol *O*-methyltransferase. *Biochim Biophys Acta* 1251: 1-10, 1995.
- 21 Zhu BT, Ezell EL and Liehr JG: Catechol-*O*-methyltransferase-catalyzed rapid *O*-methylation of mutagenic flavonoids. Metabolic inactivation as a possible reason for their lack of carcinogenicity *in vivo*. *J Biol Chem* 269: 292-299, 1994.

- 22 Zhu BT: Catechol-*O*-methyltransferase (COMT)-mediated methylation metabolism of endogenous bioactive catechols and modulation by endobiotics and xenobiotics: importance in pathophysiology and pathogenesis. *Curr Drug Metab* 3: 321-349, 2002.
- 23 Lotta T, Vidgren J, Tilgmann C, Ulmanen I, Melén K, Julkunen I and Taskinen J: Kinetics of human soluble and membrane-bound catechol *O*-methyltransferase a revised mechanism and description of the thermolabile variant of the enzyme. *Biochemistry* 34: 4202-4210, 1995.
- 24 Esteller M, Tortola S, Toyota M, Capella G, Peinado MA, Baylin SB and Herman JG: Hypermethylation-associated inactivation of *p14(ARF)* is independent of *p16(INK4a)* methylation and *p53* mutational status. *Cancer Res* 60: 129-133, 2000.
- 25 Herman JG, Graff JR, Myöhänen S, Nelkin BD and Baylin SB: Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci USA* 93: 9821-9826, 1996.
- 26 Chen J, Lipska BK, Halim N, Ma QD, Matsumoto M, Melhem S, Kolachana BS, Hyde TM and Herman MM, Apud J, Egan MF, Kleinman JE, Weinberger DR: Functional analysis of genetic variation in catechol-*O*-methyltransferase (COMT): effects on mRNA, protein, and enzyme activity in postmortem human brain. *Am J Hum Genet* 75: 807-821, 2004.
- 27 Skibola CF, Bracci PM, Paynter RA, Forrest MS, Agana L, Woodage T, Guegler K, Smith MT and Holly EA: Polymorphisms and haplotypes in the *cytochrome P450 17A1*, *prolactin*, and *catechol-*O*-methyltransferase* genes and non-Hodgkin lymphoma risk. *Cancer Epidemiol Biomarkers Prev* 14: 2391-2401, 2005.
- 28 Lavigne JA, Helzlsouer KJ, Huang HY, Strickland PT, Bell DA, Selmin O, Watson MA, Hoffman S, Comstock GW and Yager JD: An association between the allele coding for a low activity variant of catechol-*O*-methyltransferase and the risk for breast cancer. *Cancer Res* 57: 5493-5497, 1997.
- 29 Goodman MT, McDuffie K, Kolonel LN, Terada K, Donlon TA, Wilkens LR, Guo C and Le Marchand L: Case-control study of ovarian cancer and polymorphisms in genes involved in catecholestrogen formation and metabolism. *Cancer Epidemiol Biomarkers Prev* 10: 209-216, 2001.
- 30 Suzuki M, Mamun MR, Hara K, Ozeki T, Yamada Y, Kadokawa T, Honda H, Yanagihara Y, Ito YM, Kameyama S, Ohta N, Hosoi T, Arai T, Sawabe M, Takeuchi T, Takahashi S and Kitamura T: The Val158Met polymorphism of the *catechol-*O*-methyltransferase* gene is associated with the PSA-progression-free survival in prostate cancer patients treated with estramustine phosphate. *Eur Urol* 48: 752-759, 2005.
- 31 Issa JP: GpG-island methylation in aging and cancer. *Curr Top Microbiol Immunol* 249: 101-118, 2000.
- 32 Asaka M, Sugiyama T, Nobuta A, Kato M, Takeda H and Graham DY: Atrophic gastritis and intestinal metaplasia are strongly related to *Helicobacter pylori* infection and not to aging in Japan: results of a large multi-center study. *Helicobacter* 6: 294-299, 2001.
- 33 Naito Y and Yoshikawa T: Molecular and cellular mechanisms involved in *Helicobacter pylori*-induced inflammation and oxidative stress. *Free Radic Biol Med* 33: 323-336, 2002.

*Received January 28, 2009**Revised May 7, 2009**Accepted May 15, 2009*