Host Genetic Factors, Related to Inflammatory Response, Influence the CpG Island Methylation Status in Colonic Mucosa in Ulcerative Colitis

TOMOMITSU TAHARA[1], TOMOYUKI SHIBATA[1], MASAKATSU NAKAMURA[1], MASAAKI OKUBO[1], HIROMI YAMASHITA[1], DAISUKE YOSHIOKA[1], JOH YONEMURA[1], YOSHIO KMIYA[1], TAKAMITSU ISHIZUKA[1], HIROSHI FUJITA[1], MITSUO NAGASAKA[1], HIDETO YAMADA[2], ICHIRO HIRATA[1] and TOMIYASU ARISAWA[2]

[1]*Department of Gastroenterology, Fujita Health University School of Medicine, Toyoake, Japan;*
[2]*Department of Gastroenterology, Kanazawa Medical University, Ishikawa, Japan*

Abstract. *Background: CpG island hypermethylation (CIHM) in gene promoter is frequently observed in the colonic mucosa in ulcerative colitis (UC), and is strongly involved in UC-associated colorectal carcinogenesis (CRC). The influence of common single nucleotide polymorphisms (SNPs) related to inflammatory immune response on the individual susceptibility to CIHM status in the non-neoplastic rectal mucosa in UC patients was evaluated. Patients and Methods: Ten candidate SNPs, multidrug resistance 1 (MDR1) 3435 (C>T), regulated upon activation, normal T-cell expressed and secreted (RANTES)-28 (C>G), heat-shock protein (HSP)70-2 1267 (B>A), NADPH oxidase p22PHOX 242 (C>T), Toll-like receptor (TLR)2-196 to -174 (ins >del), CD14-159 (C>T), Mannan-binding lectin (MBL)2 codon 54 (G>A), tumor necrosis factor-α(TNF-α) -857 (C>T), interleukin-1β(IL-1β)-511 (C>T), and IL-1β -31 (T>C) were genotyped in 58 UC patients without neoplastic lesions, in relation to CIHM in the rectal mucosa of three candidate CpG (p14, p16 and E-cadherin: CDH1) loci, assessed by methylation-specific-polymerase chain reaction (MSP). High CIHM was defined as two or more CpG islands methylated. Results: The CD14-159TT genotype held a significantly higher susceptibility to CIHM of the p16 promoter (OR=3.82, 95%CI=1.06-13.79, p=0.04) A significant association was also found between the IL-1β-31TC genotype and reduced susceptibility to high CIHM (OR=0.14, 95%CI=0.22-0.94, p=0.04). The p22PHOX 242CT genotype and MBL2 codon 54 A carrier (GA+AA) were significantly associated with a lower mean number of CIHM (p=0.029, 0.046, respectively). Conclusion: CD14 -159, IL-1β-31, p22PHOX 242 and MBL2 codon 54 SNPs may influence the CIHM status in the rectal mucosa of UC patients and may therefore be substantially involved in UC- associated carcinogenesis.*

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. Current address: 7300 Brompton St., 5300 Houston TX 77025 U.S.A.

Key Words: Polymorphism, ulcerative colitis, DNA methylation, CD14, p22PHOX, MBL.

Ulcerative colitis (UC) affects the colon and rectum and typically involves the innermost lining mucosa, manifesting as continuous areas of inflammation, with no segments of normal mucosa (1). It is characterized by chronic, relapsing colonic inflammation with unknown etiology. UC is a multifactorial, polygenic disease with probable genetic heterogeneity. According to this hypothesis, different genetic backgrounds may explain the various clinical patterns of the disease (2, 3). Patients with UC are also predisposed to the development of colorectal carcinomas (CRC) compared with the average-risk population risk (4, 5). Important risk factors for developing CRC in UC are long-standing colitis (5), extensive colonic involvement (4), positive family history of CRC (6, 7), primary sclerosing cholangitis (8), and histologic evidence of active inflammation (9). The median age at diagnosis of CRC in patients with UC is significantly earlier than in sporadic CRC (10). Therefore, intensive CRC surveillance is strongly recommended for patients with UC and these risk factors. However, the accuracy of risk factors to predict neoplasias at an early stage is limited.

CpG island hypermethylation (CIHM) in gene promoters has been shown to be an important mechanism in gene silencing. In many kinds of cancer, some genes acquire aberrant methylation in their CpG islands. Meanwhile, some genes are methylated in non-neoplastic tissues by aging, and this alteration is known as age-related methylation (11, 12). In addition, it has been shown that gene methylation is closely associated with the inflammatory state of digestive organs (13-17), suggesting that gene methylation is a result

of chronic inflammation. In UC patients, a high degree of CIHM in nondysplastic mucosa has been reported in both the dysplastic and nondysplastic colonic mucosa (14), and was closely correlated with the risk of CRC (18). It is possible that CIHM is one of the mediators of UC-associated neoplasias and thus this molecular abnormality may allow the identification of effective markers to stratify the risk of progression to cancer in UC.

As exogenous carcinogens, generated reactive oxygen, and host genetic differences may influence the CHIM status (19), it is possible that continuous severe inflammation in the colonic musosa could accelerate CIHM in UC patients. However, there is considerable variation in the extent of colonic inflammation among UC patients and not all of them show same the CIHM status. This suggests that some genetic difference may contribute to this process, and may substantially reflect the future risk for developing CRC. To clarify this concept, the association between candidate single-nucleotide polymorphisms (SNPs), related to inflammatory immune response, oxidative stress, or the xenobiotic pathway, with the CIHM status of three tumor suppressor genes (*p16, p14* and *CDH1*) in the rectal mucosa in UC were evaluated.

Patients and Methods

Patients and samples. A total of 58 patients, 34 male and 24 female with UC were enrolled in the study.

The average age was 40.7±14.2 years and the median clinical disease duration was 9.5±7.7 years. Rectal inflammatory mucosal specimens were obtained from all the patients, during colonoscopic biopsy, and preserved at –80˚C until use. All the diagnoses were confirmed by histopathological examinations and no evidence of dysplasia or neoplasia was found in any of the cases. The Ethics Committee in Fujita Health University approved the specimen collection procedures.

Bisulfite modification and methylation-specific PCR. DNA was extracted from the specimens by protein precipitation methods and bisulfite modification was performed according to the protocol recommended by the manufacture. (BisulFast Kit Toyobo Co., Osaka, Japan). Three candidate promoter CpG islands, *p16, p14* and *CDH1* were chosen to detect CIHM. Methylation-specific PCR (MSP) was carried out with the following primers: *p16* methylated forward (MF), 5'-ttattagagggtggggcggatcgc-3' and *p16* methylated reverse (MR), 5'-gaccccgaaccgcgaccgtaa-3' , which amplified a 150-bp product; *p16* unmethylated forward (UF), 5' -ttattagagggtggggtggattgt-3' and *p16* unmethylated reverse (UR), 5'-caaccccaaaccacaaccataa-3', which amplified a 151-bp product (20); *p14* MF, 5'-gtgttaaagggcggcgtagc-3' and *p14* MR, 5'-aaaaccctcactcgcgacga-3', which amplified a 122-bp product; *p14* UF, 5'-tttttggtgttaaagggtggtgtagt-3' and *p14* UR, 5'-cacaaaaaccctcactcacaacaa-3' , which amplified a 132-bp product (21); *CDH1* MF, 5'-ttaggttagagggttatcgcgt-3' and *CDH1* MR, 5' -taactaaaaattcacctaccgac-3' , which amplified a 115-bp product and *CDH1* UF, 5'-taattttaggttagagggttattgt-3' and *CDH1* UR, 5'-cacaaccaatcaacaacaca-3', which amplified a 97-bp product (20). Annealing temperature and times were determined using DNA from the peripheral blood of a young individual and DNA was methylated with *SssI* methylase (New England BioLabs Inc., Beverly, MA, USA). The MSP chain reaction was carried out in a volume of 20 μl containing 20 ng of bislufite-modificated DNA.

The DNA was denatured at 95˚C for 5 min, followed by 33-35 cycles at 95˚C for 30 s, 57-69˚C for 1 min, and 72˚C for 1 min with a final extension at 72˚C for 5 min. The MSP reactions were conducted using EX Taq HS (Takara Bio, Inc., Shiga, Japan). The PCR products (10 μl) were separated by electrophoresis in 2.5% agarose gels and visualized by UV illumination using ethidium bromide staining. CIHM was defined as the presence of a positive methylation band showing signals approximately equal to or greater than that of the size marker (10 ng/μl: 100 bp DNA Ladder, Takara Bio) regardless of the presence of unmethylated bands. Samples giving faint positive signals were analyzed twice further and only samples with a consistent positive methylation band were considered as CHIM. In addition, the fluorescent intensities of the methylated bands of randomly selected CHIM samples were measured using a digital densitometer (Lane Analyzer, Atto, Tokyo, Japan) and confirmed that the fluorescent intensities of all the methylated bands were approximately equal to or greater than that of the size marker (data not shown). High CIHM was defined as the presence of two or more methylated CpG islands.

SNP selection and genotyping. To evaluate the association between CIHM status and host genetic factors, ten candidate common SNPs, that would be related to inflammatory immune response, oxidative stress or the xenobiotic pathway were selected. The candidate genotypes included multidrug resistance 1 (*MDR1*) 3435(C>T), regulated upon activation, normal T-cell expressed and secreted (*RANTES*) -28(C>G), heat-shock protein (HSP) 70-2 1267 (B>A), NADPH oxidase *p22PHOX* 242(C>T), Toll-like receptor (TLR)2 -196 to -174(ins >del), *CD14* -159(C>T), mannan-binding lectin (MBL)2 codon 54 (G>A), tumor necrosis factor-α(TNF-α)-857 (C>T), interleukin-1β(IL-1β)-511(C>T), and IL-1β-31(T>C) SNPs. All the genotypes were determined by using DNA extracted from peripheral blood. Except for the TLR2 -196 to -174 (ins >del) polymorphism, genotyping was performed by the PCR–restriction fragment length polymorphism (PCR–RFLP) method (22-28). For the TLR2 -196 to -174(ins >del) polymorphism, PCR was used (29).

Statistical analyses. CIHM and genotype frequencies were calculated by direct counting. Association between the CIHM status and individual genotypes was determined by the Chi-square test using a 2×2 contingency table, and the odds ratio (OR) with 95% confidence interval (CI) was calculated. The mean number of CIHM was assessed in different genotypes by using Student's *t*-test. A *p*-value <0.05 was considered statistically significant.

Results

All 58 rectal mucosa samples were available for MSP. Overall, CIHM was found in 30 (51.7%) for *p16*, 25 (43.1%) for *p14*, and 23 (39.7%) patients for *CDH1*. High CIHM was also found in 26 (44.8%) patients. The CHIM of all three genes and high CIHM were not significantly associated with age or gender (all *p*-values> 0.20, data not shown). Table I shows the associations of CIHM status of the three promoter CpG islands and high CIHM with the candidate genotypes.

Table I. *Association between genetic polymorphisms and CIHM status of colonic mucosa in UC patients*.

Variables(n)	*p16*		*p14*		*CDH1*		High CIHM	
	UM	M	UM	M	UM	M	(–)	(+)
MDR1 3435(C>T)								
CC	6	8	10	4	10	4	8	6
CT	17	17	17	17	19	15	18	16
TT	6	4	7	3	7	3	6	4
RANTES -28(C>G)								
CC	22	24	25	21	27	19	23	23
CG	6	5	8	3	8	3	8	3
GG	1	0	1	0	1	0	1	0
HSP70-2 1267 (B>A)								
BB	25	27	31	21	33	19	29	23
AB	3	2	3	2	3	2	3	2
p22PHOX 242 (C>T)								
CC	22	27	26	23	29	20	24	25
CT	6	1	7	0	5	2	7	0
TLR2 -196 to -174(ins >del)								
ins ins	11	13	13	7	14	6	12	8
ins del	13	3	16	10	15	11	15	11
del del	4	9	5	2	7	0	5	2
CD14 -159(C>T)								
CC	2	4	3	3	3	3	2	4
CT	14	5	14	5	13	6	15	4
TT	11	15	16	10	18	8	14	12
MBL2 codon 54 (G>A)								
GG	12	18	15	15	16	14	14	16
GA	17	8	19	6	18	7	18	7
AA	0	2	0	2	1	1	0	2
TNF-α-857 (C>T)								
CC	21	20	24	17	27	14	23	18
CT	26	8	10	4	9	5	9	5
TT	2	1	0	3	0	3	0	3
IL-1β-511(C>T)								
CC	8	11	10	9	10	9	10	9
CT	18	15	20	13	22	11	19	14
TT	2	3	3	2	3	2	2	3
IL-1β-31(T>C)								
TT	5	5	6	4	7	3	7	3
TC	18	16	21	13	21	13	20	14
CC	4	8	5	7	6	6	3	9

p16 M
p22PHOX 242CT: OR=0.14, 95% CI=0.02-1.21, p=0.07
CD14 -159CC: OR=5.60, 95% CI=0.77-40.62, p=0.09
CD14 -159TT: OR=3.82, 95% CI=1.06-13.79, p=0.04

High CIHM
CD14 -159CC: OR=7.50, 95% CI=1.00-56.79, p=0.05
CD14 -159TT: OR=3.21, 95% CI=0.84-12.35, p=0.09
IL-1β-31TT: OR=0.23 95% CI=0.05-1.02, p=0.053
IL-1β-31TC: OR=0.14, 95% CI=0.22-0.94, p=0.04.

UM, Unmethylated; M, methylated. High CIHM was defined as two or more CpG islands methylated. All analysis was by the Chi-square test. *HSP70-2, p22PHOX, TLR2, CD14, MBL2, IL-1β*-511, and *IL-1β*-31polymorphisms could not be genotyped for 1, 2, 5, 7, 1, 1 and 2 patients, respectively.

HSP70-2, p22PHOX, TLR2, CD14, MBL2, IL-1β-511, and *IL-1β*-31 polymorphisms could not be genotyped for 1, 2, 5, 7, 1, 1 and 2 patients, respectively. Since age and gender were not associated with CIHM status, these associations were assessed by the Chi-square test using a 2×2 contingency table.

In the comparison of genotype frequencies, the *CD14* -59TT genotype had a significantly higher susceptibility to CIHM of the p16 promoter (OR=3.82, 95% CI=1.06-13.79, p=0.04), while the CC genotype was also weakly associated with higher susceptibility to *p16* promoter CIHM. A weak

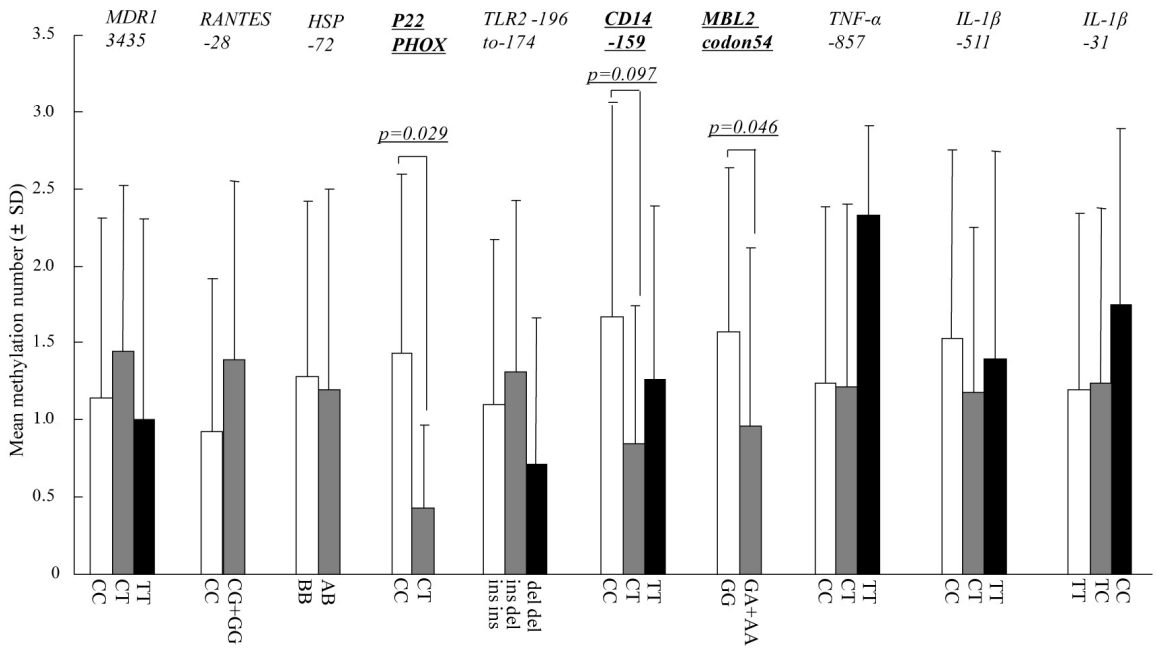

Figure 1. *Association between methylation number, expressed as mean±SD and candidate SNPs. Statistical analysis was performed by the Student's t-test. Since the number of GG genotype of RANTES -28, and AA genotype of MBL2 codon 54 were low, they were considered as G carriers (CG+GG) and A carriers (GA+AA), respectively.*

association between *p22PHOX* 242CT genotype and CIHM of the *p16* promoter was also found. Although no association was found between the selected SNPs and CIHM of the *p14*, and *CDH1* promoters, marginally significant association was found between the *IL-1β*-31TC genotype and reduced susceptibility to high CIHM (OR=0.14, 95% CI=0.22-0.94, p=0.04). A similar trend was also observed for the *IL-1β*-31TT genotype. A borderline association was also found between the *CD14* -159CC genotype (OR=7.50, 95% CI=1.00-56.79, p=0.05), and there was a weak trend for the *CD14* -159TT genotype and increased susceptibility to high CIHM. Figure 1 shows the association between the mean number of CIHM and the different genotypes. The *p22PHOX* 242CT genotype was significantly associated with a lower mean number of CIHM than the CC genotype (1.43±1.16 *vs.* 0.43±0.54, p=0.029).

In addition, the *MBL2* codon 54 A carrier (GA+AA) was also significantly associated with a lower mean number of CIHM than the GG genotype (1.57±1.07 *vs.* 0.96±1.16, p=0.046). The *CD14* -159CT genotype was weakly associated with a lower mean number of CIHM, when compared to the CC genotype.

Discussion

Some of the selected SNPs showed a marginally significant association with the CIHM status of the rectal mucosa in the UC patients.

The IL-1β-31TC were associated with reduced susceptibility to high CIHM. A similar trend was also found for the TT genotype compared to the CC genotype. *MBL2* codon 54 A carrier status was also significantly associated with a lower mean number of CIHM. IL-1 is an important proinflammatory cytokine (30) and MBL also plays a role in the innate immune response as a protein of the complement system (31). Both SNPs are associated with the activity or concentration of the respective protein (32-34). Since the inflammatory response is an important causative factor for gene methylation (19), both these SNPs may therefore influence the susceptibility to CIHM by altering an individual's inflammatory immune responses.

The *p22PHOX* 242CT genotype was also significantly associated with a lower mean methylation number, and showed a weak trend towards reduced susceptibility to CIHM of *p16*. p22PHOX is a component of the NADPH oxidase system, which transfers electrons within several subunits of NADPH oxidase and therefore plays an important role in the process of superoxide formation, as demonstrated by increases in reactive oxygen species being associated with increased levels of *p22PHOX* mRNA (35). The C242T polymorphism is thought to reduce the activation of NADPH oxidase and therefore confer protection against the superoxide related inflammatory state (36). Therefore, an association between lower methylation number and the 242CT genotype seems to be mechanistically reasonable.

As for *CD14* -159 (C>T) SNP, the -159TT genotype had a significantly higher susceptibility to CHIM, while the CC

genotype also weakly associated with CIHM of the *p16* promoter. The *CD14* -159CC and *CD14* -159TT genotypes also showed a borderline association and a weak trend, respectively, towards increased susceptibility to high CIHM while the *CD14* -159CT genotype was weakly associated with a lower mean number of CIHM, compared to the CC genotype. Thus both the CC and TT genotypes may increase and the CT genotype reduce the susceptibility to CIHM. Speculatively this polymorphism may be in linkage disequilibrium with other polymorphisms elsewhere in the *CD14* gene, demonstrating biologically relevant variability for influencing the CIHM status, or the possibility exists that this polymorphism is in linkage disequilibrium with a genetic variation of another gene located near the *CD14* gene that is related to susceptibility to CIHM.

In the present cohort of UC patients, CIHM were found at a relatively high frequency in the non-neoplastic colonic mucosa (51.7% for *p16*, 3.1% for *p14*, and 39.7% for *CDH1*). This was possibly due to the long median clinical disease duration (9.5±7.7 years), suggesting that these individuals might be at especially high risk for developing CRC. The demonstrated associations indicate that some host genetic factors may influence the CIHM status and long-term outcome of UC and may meaningfully predict patients who may be at risk of developing CRC. However, whether the associations directly contribute to the risk of CRC is unclear, because the present cohort did not include CRC patients, therefore further well-designed study will be needed to confirm the result and evaluate the clinical utility in risk prediction of CRC in UC patients.

References

1 Head KA and Jurenka JS: Inflammatory bowel disease Part I: ulcerative colitis-pathophysiology and conventional and alternative treatment options. Altern Med Rev *8*: 247-283, 2003.

2 Lakatos L and Lakatos PL: Etiopathogenesis of inflammatory bowel diseases. Orv Hetil *144*: 1853-1860, 2003.

3 Hugot JP: Inflammatory bowel disease: causes and consequences. Best Pract Res Clin Gastroenterol *18*: 447-449, 2004.

4 Ekbom A, Helmick C, Zack M and Adami HO: Ulcerative colitis and colorectal cancer. A population-based study. N Engl J Med *323*: 1228-1233, 1990.

5 Eaden JA, Abrams KR and Mayberry JF: The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut *48*: 526-535, 2001.

6 Askling J, Dickman PW, Karlen P, Brostrom O, Lapidus A, Lofberg R and Ekbom A: Family history as a risk factor for colorectal cancer in inflammatory bowel disease. Gastroenterology *120*: 1356-1362, 2001.

7 Nuako KW, Ahlquist DA, Mahoney DW, Schaid DJ, Siems DM and Lindor NM: Familial predisposition for colorectal cancer in chronic ulcerative colitis: a case-control study. Gastroenterology *115*: 1079-1083, 1998.

8 Jayaram H, Satsangi J and Chapman RW: Increased colorectal neoplasia in chronic ulcerative colitis complicated by primary sclerosing cholangitis: fact or fiction? Gut *48*: 430-434, 2001.

9 Rutter MD, Saunders BP, Wilkinson KH, Rumbles S, Schofield G, Kamm MA, Williams CB, Price AB, Talbot IC and Forbes A: Cancer surveillance in longstanding ulcerative colitis: endoscopic appearances help predict cancer risk. Gut *53*: 1813-1816, 2004.

10 Bernstein CN, Blanchard JF, Kliewer E and Wajda A: Cancer risk in patients with inflammatory bowel disease: a population-based study. Cancer *91*: 854-862, 2001.

11 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 *4*: 536-540, 1994.

12 Ahuja N, Li Q, Mohan AL, Baylin SB and Issa JP: Aging and DNA methylation in colorectal mucosa and cancer. Cancer Res *23*: 5489-5494, 1998.

13 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.

14 Issa JP, Ahuja N, Toyota M, Bronner MP and Brentnall TA: Accelerated age-related CpG island methylation in ulcerative colitis. Cancer Res 61: 3573-3537, 2001.

15 Bian YS, Osterheld MC, Fontolliet C, Bosman FT and Benhattar J: p16 inactivation by methylation of the *CDKN2A* promoter occurs early during neoplastic progression in Barrett's esophagus. Gastroenterology *122*: 1113-1121, 2002.

16 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.

17 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.

18 Garrity-Park MM, Loftus EV Jr., Sandborn WJ, Bryant SC and Smyrk TC: Methylation status of genes in non-neoplastic mucosa from patients with ulcerative colitis-associated colorectal cancer. Am J Gastroenterol *105*: 1610-1619, 2010.

19 Issa JP: GpG-island methylation in aging and cancer. Curr Top Microbiol Immunol *249*: 101-118, 2000.

20 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.

21 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.

22 Drozdzik M, Bialecka M, Mysliwiec K, Honczarenko K, Stankiewicz J and Sych Z: Polymorphism in the P-glycoprotein drug transporter *MDR1* gene: a possible link between environmental and genetic factors in Parkinson's disease. Pharmacogenetics *13*: 259-263, 2003.

23 Liu H, Chao D, Nakayama EE, Taguchi H, Goto M, Xin X, Takamatsu JK, Saito H, Ishikawa Y, Akaza T, Juji T, Takebe Y, Ohishi T, Fukutake K, Maruyama Y, Yashiki S, Sonoda S, Nakamura T, Nagai Y, Iwamoto A and Shioda T: Polymorphism in *RANTES* chemokine promoter affects HIV-1 disease progression. Proc Natl Acad Sci USA *96*: 4581-4585, 1999.

24 Fekete A, Treszl A, Tóth-Heyn P, Vannay A, Tordai A, Tulassay

T and Vásárhelyi B: Association between heat-shock protein 72 gene polymorphism and acute renal failure in premature neonates. Pediatr Res *54*: 452-455, 2003.

25 Tahara T, Shibata T, Wang F, Nakamura M, Okubo M, Yoshioka D, Sakata M, Nakano H, Hirata I and Arisawa T: Association of polymorphism of the p22PHOX component of NADPH oxidase in gastroduodenal diseases in Japan. Scand J Gastroenterol *44*: 296-300, 2009.

26 Hubacek JA, Rothe G, Pit'ha J, Skodova Z, Stanek V, Poledne R and Schmitz G: C(-260)→T polymorphism in the promoter of the CD14 monocyte receptor gene as a risk factor for myocardial infarction. Circulation *99*: 3218-3220, 1999.

27 Gomi K, Tokue Y, Kobayashi T, Takahashi H, Watanabe A, Fujita T and Nukiwa T: Mannose-binding lectin polymorpism is a modulating factor in repeated respiratory infections. Chest *126*: 95-97, 2004.

28 Sugimoto M, Furuta T, Shirai N, Nakamura A, Xiao F, Kajimura M, Sugimura H and Hishida A: Different effects of polymorphisms of tumor necrosis factor-alpha and interleukin-1 beta on development of peptic ulcer and gastric cancer. J Gastroenterol Hepatol *22*: 51-59, 2007.

29 Tahara T, Arisawa T, Wang F, Shibata T, Nakamura M, Sakata M, Hirata I and Nakano H: Toll-like receptor 2 -196 to 174del polymorphism influences the susceptibility of Japanese people to gastric cancer. Cancer Sci *98*: 1790-1794, 2007.

30 Dinarello CA: Biologic basis for interleukin-1 in disease. Blood *87*: 2095-2147, 1996,

31 Turner MW and Hamvas RMJ: Mannose-binding lectin: structure, function, genetics and disease associations. Rev Immunogenet *2*: 305-322, 2000.

32 Hwang IR, Kodama T, Kikuchi S, Sakai K, Peterson LE, Graham DY and Yamaoka Y: Effect of interleukin 1 polymorphisms on gastric mucosal interleukin 1beta production in *Helicobacter pylori* infection. Gastroenterology *123*: 1793-1803, 2002.

33 Wallace JL, Cucala M, Mugridge K and Parente L: Secretagogue-specific effects of interleukin-1 on gastric acid secretion. Am J Physiol *261*: G559-564, 1991.

34 Eisen DP and Minchinton RM: Impact of mannose-binding lectin on susceptibility to infectious diseases. Clin Infect Dis *37*: 1496-1505, 2003.

35 Babior BM: NADPH oxidase: an update. Blood *93*: 1464-1476, 1999.

36 Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R and Channon KM: Functional effect of the C242T polymorphism in the NAD(P)H oxidase *p22PHOX* gene on vascular superoxide production in atherosclerosis. Circulation *102*: 1744-1747, 2000.

Received December 13, 2010
Revised February 20, 2011
Accepted February 21, 2011