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위암 환자에서 Glutathione S-transferases (*GSTM1, GSTT1, GSTP1*) 및 N-acetyltransferase 2 유전자 다형성 분포

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Glutathione S-transferases (*GSTM1, GSTT1* and *GSTP1*) and N-acetyltransferase 2 Polymorphisms and the Risk of Gastric Cancer

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Objectives : Polymorphisms of genes from glutathione S-transferases (*GSTs*) and N-acetyltransferase 2 (*NAT2*) have been associated with increased susceptibility to various cancers. Previous results showed that East Asians such as Koreans, Japanese and Chinese have a much higher frequency of the *GSTM1* and *GSTT1* null genotypes and *NAT2* rapid acetylator type. Therefore, we investigated the association between the polymorphic types of *GSTs* (*GSTM1, GSTT1, GSTP1*) and *NAT2* and the incidence of gastric cancer which is one of the most prevalent cancers among the East Asians.

Methods : It was performed in a case-control study consisting of 238 healthy subjects and 108 cancer patients (54 distal and 54 proximal carcinomas). We also evaluated the association between *GSTs* and *NAT2* and the risk factors for gastric cancer such as alcohol consumption, smoking, *H. pylori* infection, family history of gastric cancer, and tumor location.

Results : In our study, the percentage of cases whose hometown was rural was higher than those of controls (odds ratio (OR) =2.88; 95% CI=1.72-4.76), and the frequency of the lower socio-economic status increased significantly in patients (OR=2.53; 95% CI=1.59-4.02). There was no significant difference in the GST polymorphic types between the cases and controls. However, *NAT2* rapid or intermediate acetylator types were frequently detected in the cases with family history of gastric cancer (OR=1.92; 95% CI=1.79-26.0).

Conclusions : These results suggest that the hometown and socio-economic status are important environmental factors for gastric carcinogenesis, and *NAT2* polymorphic types could be associated with familial gastric carcinoma.

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Key words : Gastric Cancer, Korean, Glutathione-S-transferase, N-acetyltransferase 2, Genetic Polymorphism

INTRODUCTION

The ability to detoxify exogenous carcinogens may influence a disease following exposure. Glutathione S-transferases (*GSTs*) and N-acetyltransferases (*NATs*) are good candidates for investigation including those involved in xenobiotic metabolism. *GST* is a complex super gene family that catalyzse the neutrophilic attack of glutathione on a wide range of hydrophilic electrophiles [1]. *NAT* can modulate drug activity and detoxify carcinogens through the acetylation of aromatic amines and hydrazines [2]. Of the two types of *NATs*, that of *NAT2* is better known, due to its capability to metabolize aromatic amines.

GSTs and *NAT2* have been shown to be polymorphically distributed and are therefore, of special interest in molecular epidemiological studies. The percentage of individuals who inherited susceptibility to chemical carcinogenesis due to *GSTs* and *NAT2* varies significantly among distinct ethnic groups. Some of the polymorphic genotypes coding for the enzymes have been shown to reduce enzyme activity [3-5], and may have an

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increased risk in developing specific types of cancer. The homozygous null *GSTM1* genotype has been reported to have some association with the pathogenesis of lung cancer and bladder cancer [6]. *GSTT1* null type has been suggested as a risk factor in colon cancer [7], and the *GSTP1* polymorphic genotype has been shown to be related to prostate cancer risk [8]. A study has suggested that individuals with specific *NAT2* genotypes may be at increased risk for colorectal cancer [9]. The association between genetic polymorphism and the development of cancer indicates the increased need to confirm the combined effects of polymorphic genes on cancer susceptibility.

Despite the decline in the number of cases, gastric cancer remains one of the leading causes of death in Korea and other East-Asian countries such as Japan and China. The etiology of gastric cancer is not well known, although some factors including alcohol consumption, cigarette smoking, and *Helicobacter pylori* infection have been found to be associated with the development of gastric cancer. There is relatively little known about how genetic factors might influence gastric cancer risk, but based on the roles that their expression products play in the metabolism of carcinogens, the polymorphic *GSTs* or *NAT2* genes are good candidates as susceptible genes. *GSTM1* and *GSTT1* are expressed at relatively high levels in many cell types along the human gastrointestinal tract [10]. Therefore, it is estimated that these enzymes play an important role in the protection against carcinogens and other xenobiotics. Interestingly, it was reported that the *GSTM1* null genotype might influence p53 genomic instability in human gastric cancer [11]. Therefore, the relationship between these genetic polymorphisms and individual susceptibility to the gastric carcinogenesis needs to be explored.

Interestingly, East Asians have been showing a much higher frequency of the *GSTM1* and *GSTT1* null genotypes and *NAT2* rapid acetylator type, and gastric cancer rates in this area are among the highest in the world. Therefore, it needs to be determined whether these polymorphic types may contribute to a predisposition to environmentally related carcinogenesis in certain population. The contribution of *GSTs* and *NAT2* genotypes to susceptibility of the risk of gastric cancer, and their interaction with clinicopathological characteristics are still unclear. In this study, we evaluated the association between the polymorphic types of *GSTs* (*GSTM1*, *GSTT1*, and *GSTP1*) and *NAT2*, and the risk factors for gastric cancer such as, alcohol consumption, smoking, *H. pylori* infection, family history of gastric cancer, and tumor location in a case-control study.

MATERIALS and METHODS

Study Population and Genomic DNA Purification

The healthy group included 238 subjects who had visited Daegu Catholic University hospital for general health check-ups. Control subjects had no current or previous diagnosis of cancer. The case group included 108 patients with gastric adenocarcinoma (54 distal and 54 proximal) from Daegu, Korea. Patients were histologically diagnosed at the Daegu Catholic University hospital during the period February through December 2000. All study subjects completed a questionnaire covering medical, residential and occupational exposures as well as socio-economic status, smoking history, and alcohol consumption following informed consent. A family's socioeconomic status is based on family income, parental education level, and parental occupation. Family history of gastric cancer was decided from each first-degree relative containing parents and/or brothers diagnosed as having gastric cancer.

Genomic DNA was isolated from peripheral leukocytes by proteinase K digestion and phenol-chloroform method [12]. The amount of DNA and its purity were determined by spectrophotometric measurement and an aliquot (about 200 ng of DNA) from each sample was used for the polymerase chain reaction.

Analyses of *GSTM1*, *GSTT1* and *GSTP1* Genotypes

The presence of the wild type and/or the null alleles of *GSTM1* and *GSTT1* was tested by multiplex PCR together with coamplification of a fragment of the *β-globin* gene as a positive control [6]. Genes were amplified in a reaction buffer containing 2.5 mM MgCl2, I U Taq DNA polymerase (Neurotics, Daejon, Korea) and 200 ng of DNA. PCR products were electrophoresed on a 2% agarose gel. The absence of a 215-bp band indicates the *GSTM1* null genotype; the absence of a 473-bp band indicates the *GSTT1* null genotype. The *GSTM1* primers were 5'-GAACTCCCT-GAAAAGCTAAAGC (sense) and 5'-GTTGGGCT-CAAATATACGGTGG (antisense). The *GSTT1* primers were 5'-TTCCTTACTGGTCCTCACATCTC (sense) and 5'-TCACCGGATCATGGCCAGCA (antisense). The primers for *β-globin* were 5'-CAACTTCATCCACGTTCACC (sense) and 5'-GAGAGCCAAGGACAGGTAC (anitsense).

The polymorphism of the *GSTP1* gene has been found to have an A-to-G transition at codon 104 [13]. We analyzed this by PCR-RFLP and determined the genotype for each subject as described by Harries et al. [14] with some modifications. The *GSTP1* primers were 5'-ACCCCAGGGCTCTATGGGAA (sense) and 5'-TGAGGGCACAAGAAGCCCCT (antisense). The 176 bp PCR product was digested with 3 U *BsmA*1 (New England Biolabs, Beverly, USA) at 55℃ for 16 h. The resulting fragments were visualized on 3% agarose gels and 20% polyacrylamide gels, and the presence of variants *GSTP1A* and *GSTP1G* was assessed. A predominant homozygote (*AA*) revealed only 176 bp band; a heterozygote (*AG*) revealed to show 176, 91, and 85 bp bands; and a rare homozygote (*GG*) digested

Table 1. Distribution of selected variables in case and control groups

Characteristics	Case (n=108)	Control (n=238)	OR (95% CI)
Age (Mean±S.D.)	62.0±11.0 yr	60.7±9.8 yr	
Sex: Male (%)	61.1	50	
Female (%)	38.9	50	
Education (Mean±S.D.)	7.7±4.2 yr	7.8±5.0 yr	
Refrigerator use (Mean±S.D.)	21.7±7.5 yr	19.8±5.9 yr	
Hometown: Urban (%)	24.0	47.7	2.88 (1.72-4.76)*
Rural (%)	76.0	52.3	
Socio-economic status:			
High (%)	40.7	63.4	2.52 (1.59-4.02)†
Low (%)	59.3	36.6†	

*χ^2 P=0.0001
†χ^2 P=0.0007

completely to demonstrate 91 and 85 bp bands.

Analysis of *NAT2* genotype

Molecular genotyping by conventional PCR of the three most common *NAT2* alleles, *NAT2*4, NAT2*6, NAT2*7*, (frequently referred to as *WT, M2,* and *M3*, respectively) was accomplished in order to predict *NAT2* phenotype [15]. We omitted the detection of allele *M1* because the frequency of this allele is very rare (<1% in the Japanese population) [16]. The primers for *NAT2* were 5' -CTTAATTCTCATCTCCTG- CC (sense) and 5' -AGCATGAATCACTCTGCTTC (antisense). After PCR amplification, the 610 bp products were digested with *Taq*I at 65℃ and *Bam*HI at 37℃ to acquire three *NAT2* alleles [16]. Digestion with *Taq*I produced 376, 170 and 64 bp fragments of alleles *NAT2*4* and *NAT2*7*, but only 376 and 234 bp fragments in the case of allele *NAT2*6*. *Bam*HI could digest the 610 bp PCR fragments of alleles *NAT2*4* and *NAT2*6*, yielding 332 and 278 bp fragments, but not allele *NAT2*7*. Phenotypes were determined by a combination of two alleles and were classified as the following: genotype *NAT2*4/*4* is rapid; genotypes *NAT2*4/*6* and *NAT2*4/*7* are intermediate; and genotypes *NAT2*6/*6*, *NAT2*6/*7*, and *NAT2*7/*7* are slow.

Statistical Analysis

Subjects were described according to basic sociodemographic factors and clinicopathological characteristics of gastric cancer. Patients who indicated that they had stopped smoking or drinking alcohol within the past 6 months were classified as current smokers or current alcohol drinkers. The distribution of selected variables and relative associations between cases and controls were assessed by calculating odds ratios (OR) and 95% confidence intervals (95% CI). Corresponding x^2 tests were carried. We used multiple logistic regressions to determine ORs using the SAS software package for Windows.

RESULTS

The distribution of selected variables in the cases and controls

Table 1 shows the distribution of age, sex, education, and refrigerator use to compare the characteristics of cases and controls. The mean ages were 62 years for cancer patients and 60.7 years for controls. Some interesting differences were noted in the hometown and socio-economic status between the two groups. Among the patients, those who grew up in rural places demonstrated a higher percentage as compared with those in urban areas (OR=2.88; 95% CI=1.72-4.76). The frequency of the lower socio-economic status increased significantly in patients as compared in controls (OR=2.53; 95% CI=1.59-4.02).

The prevalence of *GSTs* and *NAT2* polymorphic types in the cases and controls is given in Table 2. The results showed that the polymorphic types between the two groups did not significantly differ. The distribution of the combined polymorphic types showed no statistically significant differences (data not shown).

Table 2. GSTs and NAT2 frequencies in the case and control groups

Polymorphic types		Cases (%) n=108	Controls (%) n=238
GSTM1	positive	48 (44.4)	104 (43.7)
	null	60 (55.6)	134 (56.3)
GSTT1	positive	63 (58.3)	119 (50)
	null	45 (41.7)	119 (50)
GSTP1	*AA*	66 (61.1)	158 (66.4)
	AG	38 (35.2)	74 (31.1)
	GG	4 (3.7)	6 (2.5)
NAT2	Rapid	51 (47.2)	106 (44.5)
	Intermediate	45 (41.7)	98 (41.2)
	Slow	12 (11.1)	34 (14.3)

When the subjects were organized by age, there was a trend of an increase in the *GSTM1* null genotype frequency with a decrease in the age of the patients, but this is not statistically significant. There was no significant difference whether the cases and controls had been exposed to cigarette smoking, alcohol consumption, or *H. pylori* infection (Table 3). However, patients with familial gastric carcinoma showed rapid or intermediate acetylator types more frequently than those without family history of gastric carcinoma (OR, 1.92; 95% CI=1.79-26.0). We determined the interactions between polymorphic types and the risk of gastric cancer according to tumor location, and Lauren classification of cancer patients. There was no significant difference according to these variables (Table 4).

DISCUSSION

It is under investigation whether the *GSTs* and *NAT2* polymorphic types can modify the risks associated with exposure to carcinogens. The information of ethnic distribution would be useful in epidemiological studies. In this study, the prevalence of *GSTM1* and *GSTT1* deletion in healthy subjects is 56.3% and 50%, respectively. The percentage of *GSTP1AG* or *GG* variants in our study was 33.6%, and *NAT2* slow acetylator type was 14.3%. These

Table 3. The distribution of polymorphic types according to selected variables in case and control groups

Variables	n		*GSTM1* null (%)		*GSTT1* null (%)		*GSTP1 AG* or *GG* (%)		*NAT2* Slow (%)	
	Case	Control	Case	Control	Case	Control	Case	Control	Case	Control
Age groups										
≤50 yr	16	92	75.5	55.4	37.5	50.0	37.5	31.5	18.8	6.5
51-60 yr	25	72	64.0	48.6	36.0	51.4	44.0	34.7	16.0	16.7
61-70 yr	43	53	51.2	62.3	44.2	47.2	37.2	39.6	11.6	20.8
≥71 yr	24	21	41.7	71.4	45.8	52.4	37.5	23.8	0	19.0
Alcohol										
Yes*	42	92	57.1	56.5	35.7	56.5	36.3	33.7	9.1	8.7
No	66	146	56.1	56.2	45.4	46.6	43.7	33.6	10.9	17.1
Smoking										
Yes†	60	104	56.7	57.1	43.3	52.4	39.1	35.2	0.9	17.6
No	48	134	56.3	54.8	39.6	47.4	43.2	31.9	9.0	18.5
H. pylori Infection										
Yes	71	130	60.6	56.5	40.9	47.3	42.2	31.3	11.3	14.5
No	37	108	51.4	55.6	40.5	52.8	35.1	38.9	8.1	13.0
Family history‡										
Yes	94	21	54.3	52.4	42.4	47.6	37.2	19.0	7.5§	14.3
No	14	217	71.4	56.7	35.7	49.8	50.0	34.6	28.6	12.4

*Current or ex-drinkers
†Current or ex-smokers
‡One or more first-degree relatives with gastric cancer
§OR=1.92 (95% CI, 1.79-25.99), $\chi 2$ P=0.005

Table 4. The distribution of polymorphic types according to clinicopathological characteristics of cases

Variables	n	*GSTM1* (%)		*GSTT1* (%)		*GSTP1* (%)		*NAT2* (%)	
		present	null	present	null	AA	*AG or GG*	Rapid or Intermediate	Slow
Tumor location									
Distal	54	46.3	53.7	59.3	40.7	59.3	40.7	87.0	13.0
Proximal	54	40.7	59.3	57.4	42.6	63.0	37.0	94.4	5.6
Lauren classification									
Intestinal	35	31.4	68.6	57.1	42.9	65.7	34.3	82.8	17.2
Mixed	23	43.5	56.5	52.2	47.8	60.9	39.1	90.9	9.1
Diffuse	50	52.0	48.0	62.0	38.0	58.0	42.0	92.2	7.8

frequencies are comparative with the previous results with other healthy Koreans [17-19], and East Asians [20-22]. The distribution of these genotypes, however, is different from other ethnic groups [22-26]. In the present study, we evaluated the association between *GSTs* and *NAT2* polymorphism and the risk factors for gastric carcinoma whose rates in the East Asians are among the highest in the world.

We investigated the distribution of some variables between the cases and controls. During the past 50 years, the number of reported cases and subsequent deaths attributed to gastric cancer has decreased worldwide [27]. Even the exact causes of the decline of gastric cancer are not well understood, dietary modifications and, possibly, fresh fruit and vitamin supplements remain one of the most important tool for the prevention of gastric cancer [28]. In our study, the percentage of cases whose hometown was rural (OR=2.88, 95% CI=1.72-4.76), and whose socio-economic status was lower (OR=2.53; 95% CI=1.59-4.02) were significantly higher than that of controls, and these results are consistent with previous data [27].

Previous reports showed that there was no association between the *GSTM1* and *GSTT1* genotypes and gastric cancer in the Japanese population [29]. Another study, however, showed that the prevalence of the *GSTT1* null genotype was higher in gastric cancer cases than in the controls [30]. The *GSTP1* genotype did not seemed to be associated with the risk of gastric cancer in the Chinese population [31]. Previous study showed that, individuals with *NAT2* rapid acetylator type are at increased risk of developing gastric carcinoma in Europeans [32], and in Koreans [19]. Another study, however, performed in the USA showed that there was no correlation between *NAT2* polymorphic types and gastric cancer [33]. In the present study, there was no significant difference in the distribution of *GSTs* or *NAT2* between the cases and controls. The discrepancies of these studies may be due to different sample sizes and dietary habits of the study groups.

Previous report showed that the frequency of the *GSTM1* null genotype in younger gastric carcinoma patients (<50 years) was higher than in controls [29]. It was also reported that the frequency of the *GSTT1* null genotype appeared to decrease with an increase in the age of colorectal cancer patients [34]. In the present study, *GSTM1* null genotype frequency increased with decreasing age of the case subjects, but, it was not statistically significant.

Since *GSTs* and *NAT2* may play an important role in the metabolism of tobacco smoke-derived carcinogens, the risk of gastric cancer associated with the polymorphisms of these enzymes may depend on the individuals' smoking status. It was reported that the susceptibility of gastric cancer in smokers was increased by their *GSTM1* null phenotype [35]. In our study, the *NAT2* type does not play a significant role in gastric cancer according to their smoking habits, and this was consistent with a previous study [36]. There was evidence that in humans, the *NAT1* plays the major role in the acetylation of benzidine [37], and its genotype may be an important genetic determinant in smoking-induced bladder cancer [15]. Therefore, it would be worth investigating whether *NAT1* polymorphic types play a role in gastric cancer cases exposed to smoking.

Chronic *H. pylori* infection associated with a higher risk of gastric carcinoma has been demonstrated repeatedly. The precise mechanism, however, as to how this microorganism induces gastric carcinogenesis, and why only a small minority of infected individuals develops gastric carcinoma remain unanswered. A previous report showed that the

null genotype for *GSTM1* was found more commonly in *H. pylori*-associated gastric carcinoma [38]. Yet another study showed that there was no association between the risk of gastric cancer infected with *H. pylori* and the *GST* genotypes [30], and this is consistent with our study.

Many studies so far have revealed that the presence of a family history of gastric carcinoma is associated with an increased risk of developing the disease in both men and women. However, little is known about the relative contributions of genetic and environmental factors to the development of familial gastric carcinoma. No previous study has shown the possible interaction in gastric cancer between the *GSTs* and *NAT2* genotypes and familial gastric cancer. We examined to see if there is a significant difference in the distribution of *GSTs* or *NAT2* according to the family history of gastric cancer patients. Interestingly, *NAT2* rapid or intermediate acetylator types were associated with a familial gastric carcinoma of the patients (OR=1.92, 95% CI=1.79-26.0). In the present study, however, there is a big difference in the numbers of patients with or without family history of gastric carcinoma. Therefore, more detailed studies need to be undertaken to better understand whether an enhanced acetylation may contribute to gastric cancer predisposition.

We analyzed the frequency of *GSTs* and *NAT2* among gastric cancer patients following stratification by the location and Lauren classification. A previous study showed that the frequency of the null *GSTM1* genotype in distal gastric cancer patients showed a statistically significant increase compared to proximal cancer [29]. Our results, however, did not show this effect.

Previous study showed that some food items such as green vegetables, pork, and soybean crud changed significantly the association of *GSTs* with the development of gastric carcinoma [39]. Recent study showed that the combination of *GSTM1* and *CYP2E1* genotypes increased the risk for diffuse-type gastric cancer [40]. Therefore, further studies need to be performed to investigate the association of other genetic, environmental, or habitual factors with the gastric carcinogenesis.

SUMMARY and CONCLUSION

From this study, it could be concluded that the rural hometown and lower socio-economic status could be important in the evocation of gastric carcinoma, but the polymorphic types of *GSTs* do not play significant role in the overall risk of gastric cancer in the study population. However, the *NAT2* rapid or intermediate acetylator types were associated with familial gastric carcinoma. To investigate the precise functions of *GSTs* and *NAT2* in gastric carcinogenesis, the metabolic pathways of some important carcinogens inside the body need to be identified clearly.

REFERENCES

1. Ketterer B. Protective role of glutathione and glutathione transferases in mutagenesis and carcinogenesis. *Mutat Res* 1988; 202: 343-361
2. Hein DW, Doll MA, Rustan TD, Gray K, Feng Y, Ferguson RJ, Grant DM. Metabolic activation and deactivation of arylamine carcinogens by recombinant human *NAT1* and polymorphic *NAT2* acetyltransferases. *Carcinogenesis* 1993; 14: 1633-1638
3. Gross M, Kruisselbrink T, Anderson K, Lang N, McGovern P, Delongchamp R, Kadlubar F. Distribution and concordance of N-acetyltransferase genotype and phenotype in an American population. *Cancer Epidemiol Biomarkers Prev* 1999; 8: 683-692
4. Pemble S, Schroeder KR, Spencer SR, Meyer DJ, Hallier E, Bolt HM, Ketterer B, Taylor JB. Human glutathione S-transferase theta (*GSTT1*): cDNA cloning and the characterization of a genetic polymorphism. *Biochem J* 1994; 300 (Pt 1): 271-276
5. Wiencke JK, Pemble S, Ketterer B, Kelsey KT. Gene deletion of glutathione S-transferase theta: correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidemiol Biomarkers Prev* 1995; 4: 253-259
6. Bell DA, Taylor JA, Paulson DF, Robertson CN. Mohler JL. Lucier GW. Genetic risk and carcinogen exposure: a common inherited defect of the carcinogen-metabolism gene glutathione S-transferase M1 (*GSTM1*) that increases susceptibility to bladder cancer. *J Natl Cancer Inst* 1993; 85: 1159-1164
7. Chenevix-Trench G, Young J, Coggan M, Board P. Glutathione S-transferase M1 and T1 polymorphisms: susceptibility to colon cancer and age of onset. *Carcinogenesis* 1995; 16: 1655-1657
8. Gsur A, Haidinger G, Hinteregger S, Bernhofer G, Schatzl G, Madersbacher S, Marberger M, Vutuc C, Micksche M. Polymorphisms of glutathione-S-transferase genes (*GSTP1, GSTM1* and *GSTT1*) and prostate-cancer risk. *Int J Cancer* 2001; 95: 152-155
9. Bell DA, Badawi AF, Lang NP, Ilett KF, Kadlubar FF, Hirvonen A. Polymorphism in the N-acetyltransferase 1 (*NAT1*) polyadenylation signal: association of *NAT1**10 allele with higher N-acetylation activity in bladder and colon tissue. *Cancer Res* 1995; 55: 5226-5229
10. de Bruin WC, Wagenmans MJ, Peters WH. Expression of glutathione S-transferase alpha, P1-1 and T1-1 in the human gastrointestinal tract. *Jpn J Cancer Res* 2000; 91: 310-316
11. Conde AR, Martins G, Saraiva C, Rueff J, Monteiro C. Association of p53 genomic instability with the glutathione S-transferase null genotype in gastric cancer in the Portuguese population. *Mol Pathol* 1999; 52: 131-134
12. Sambrook J FE, Maniatis T. Isolation of high-molecular-weight DNA from mammalian cells. In: Sambrook J, Maniatis T, editors. Molecular cloning: A laboratory manual. New York: Cold Spring Harbor Lab. Press; 1989. p. 9.16-19.22
13. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J. Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins. *J Biol Chem* 1997; 272: 10004-10012
14. Harries LW, Stubbins MJ, Forman D, Howard GC, Wolf CR. Identification of genetic polymorphisms at the glutathione S-transferase Pi locus and association with susceptibility to bladder, testicular and prostate cancer. *Carcinogenesis* 1997; 18: 641-644
15. Taylor JA, Umbach DM, Stephens E, Castranio T, Paulson D, Robertson C, Mohler JL, Bell DA. The role of N-acetylation polymorphisms in smoking-associated bladder cancer: evidence of a gene-gene-exposure three-way interaction. *Cancer Res* 1998; 58: 3603-3610

16. Mashimo M, Suzuki T, Abe M, Deguchi T. Molecular genotyping of N-acetylation polymorphism to predict phenotype. *Hum Genet* 1992; 90: 139-143
17. Kang T, El-Sohemy A, Cornelis M, Joung C, Lee H, Uhm W, Kim T, Jun J, Yoo D, Kim T, Bae S. Glutathione S-transferase Gene Polymorphisms and Systemic Lupus Erythematosus. *Korean Rheum Assoc* 2003; 10: 234-2 (Korean)
18. Nam HM KH, Kang JW, Bae JW, Choe KH, Lee KH, Kim ST, Won CW, Kim YM. A Case-Control Study on Effects of Gsnetic Polymorphisms of *GSTM1, GSTT1, CYP1A1* and *CYP2E1* on Risk of Lung Cancer. *Korean J Prev Med* 1999; 32: 123-129 (Korean)
19. Yun H, Kim J, Song Y, Park J, Lee C, Nam H, Kim H. Effect of the Genetic Polymorphism of N-acetyltransferase 2 and Diet on the Carcinogenesis of Gastric Cancer in Koreans. *J Korean Surg Soc.* 2003; 64: 459-465 (Korean)
20. Hung HC, Chuang J, Chien YC, Chern HD, Chiang CP, Kuo YS, Hildesheim A, Chen CJ. Genetic polymorphisms of *CYP2E1, GSTM1,* and *GSTT1*; environmental factors and risk of oral cancer. *Cancer Epidemiol Biomarkers Prev* 1997; 6: 901-905
21. Yamamura K, Kiyohara C, Nakanishi Y, Takayama K, Hara N. [Lung cancer risk and genetic polymorphism at the glutathione S-transferase P1 locus in male Japanese]. *Fukuoka Igaku Zasshi* 2000; 91: 203-206
22. Ellard GA. Variations between individuals and populations in the acetylation of isoniazid and its significance for the treatment of pulmonary tuberculosis. *Clin Pharmacol Ther* 1976; 19: 610-625
23. Lin HJ, Han CY, Bernstein DA, Hsiao W, Lin BK, Hardy S. Ethnic distribution of the glutathione transferase Mu 1-1 (*GSTM1*) null genotype in 1473 individuals and application to bladder cancer susceptibility. *Carcinogenesis* 1994; 15: 1077-1081
24. Kelsey KT, Spitz MR, Zuo ZF, Wiencke JK. Polymorphisms in the glutathione S-transferase class mu and theta genes interact and increase susceptibility to lung cancer in minority populations (Texas, United States). *Cancer Causes Control* 1997; 8: 554-559
25. Nelson HH, Wiencke JK, Christiani DC, Cheng TJ, Zuo ZF, Schwartz BS, Lee BK, Spitz MR, Wang M, Xu X. Ethnic differences in the prevalence of the homozygous deleted genotype of glutathione S-transferase theta. *Carcinogenesis* 1995; 16: 1243-1245
26. Curran JE, Weinstein SR, Griffiths LR. Polymorphisms of glutathione S-transferase genes (*GSTM1, GSTP1* and *GSTT1*) and breast cancer susceptibility. *Cancer Lett* 2000; 153: 113-120
27. Parker SL, Tong T, Bolden S, Wingo PA. Cancer statistics, 1997. *CA Cancer J Clin* 1997; 47: 5-27
28. Munoz N, Franceschi S. Epidemiology of gastric cancer and perspectives for prevention. *Salud Publica Mex* 1997; 39: 318-330
29. Katoh T, Nagata N, Kuroda Y, Itoh H, Kawahara A, Kuroki N, Ookuma R, Bell DA. Glutathione S-transferase M1 (*GSTM1*) and T1 (*GSTT1*) genetic polymorphism and susceptibility to gastric and colorectal adenocarcinoma. *Carcinogenesis* 1996; 17: 1855-1859
30. Setiawan VW, Zhang ZF, Yu GP, Li YL, Lu ML, Tsai CJ, Cordova D, Wang MR, Guo CH, Yu SZ, Kurtz RC. *GSTT1* and *GSTM1* null genotypes and the risk of gastric cancer: a case-control study in a Chinese population. *Cancer Epidemiol Biomarkers Prev* 2000; 9: 73-80
31. Setiawan VW, Zhang ZF, Yu GP, Lu QY, Li YL, Lu ML, Wang MR, Guo CH, Yu SZ, Kurtz RC, Hsieh CC. *GSTP1* polymorphisms and gastric cancer in a high-risk Chinese population. *Cancer Causes Control* 2001; 12: 673-681
32. Ladero JM, Agundez JA, Olivera M, Lozano L, Rodriguez-Lescure A, Diaz-Rubio M, Benitez J. N-acetyltransferase 2 single-nucleotide polymorphisms and risk of gastric carcinoma. *Eur J Clin Pharmacol* 2002; 58: 115-118
33. Boissy RJ, Watson MA, Umbach DM, Deakin M, Elder J, Strange RC, Bell DA. A pilot study investigating the role of *NAT1* and *NAT2* polymorphisms in gastric adenocarcinoma. *Int J Cancer* 2000; 87: 507-511
34. Welfare M, Monesola Adeokun A, Bassendine MF, Daly AK. Polymorphisms in *GSTP1, GSTM1,* and *GSTT1* and susceptibility to colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 1999; 8: 289-292
35. Cai L, Yu SZ, Zhang ZF. Glutathione S-transferases M1, T1 genotypes and the risk of gastric cancer: a case-control study. *World J Gastroenterol* 2001; 7: 506-509
36. Katoh T, Boissy R, Nagata N, Kitagawa K, Kuroda Y, Itoh H, Kawamoto T, Bell DA. Inherited polymorphism in the N-acetyltransferase 1 (*NAT1*) and 2 (*NAT2*) genes and susceptibility to gastric and colorectal adenocarcinoma. *Int J Cancer* 2000; 85: 46-49
37. Zenser TV, Lakshmi VM, Rustan TD, Doll MA, Deitz AC, Davis BB, Hein DW. Human N-acetylation of benzidine: role of *NAT1* and *NAT2*. *Cancer Res* 1996; 56: 3941-3947
38. Kato S, Onda M, Matsukura N, Tokunaga A, Matsuda N, Yamashita K, Shields PG. Genetic polymorphisms of the cancer related gene and Helicobacter pylori infection in Japanese gastric cancer patients. An age and gender matched case-control study. *Cancer* 1996; 77: 1654-1661
39. Park J, Song Y, Yun H, Kim H, Ahn Y, Kim S, Kim J, Kang J, Nan H, Kim Y. The Risk of Gastric Cancer in Koreans according to Smoking, Drinking, Diet, and the Genetic Polymorphisms of Glutathione S-Transferases and L-myc Protooncogene. *J Korean Cancer Assoc* 2000; 32: 997-1006
40. Suzuki S, Muroishi Y, Nakanishi I, Oda Y. Relationship between genetic polymorphisms of drug-metabolizing enzymes (*CYP1A1, CYP2E1, GSTM1,* and *NAT2*), drinking habits, histological subtypes, and p53 gene point mutations in Japanese patients with gastric cancer. *J Gastroenterol* 2004; 39: 220-230