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■ BRIEF COMMUNICATION ■

GSTM1, *GSTT1* and *GSTP1* Polymorphisms in the Korean Population

The isoenzymes of the glutathione s transferase (GST) family play a vital role in phase II of biotransformation of many substances. Using a multiplex polymerase chain reaction and a direct sequencing analysis, the frequencies of *GSTM1, GSTT1*, and *GSTP1* polymorphisms were evaluated in 1,051 Korean male subjects. We found that 53.8% of the individuals had the *GSTM1* null genotype and 54.3% had the *GSTT 1* null genotype. The genotypic distribution of *GSTP1* was Ile_{105}/Ile_{105} in 68.4%, Ile_{105}/Val_{105} in 29.1% and Val_{105}/Val_{105} in 2.5%. The most frequently observed combination of *GSTM1, GSTP1* and *GSTT1* genotypes was Null type/Ile_{105}/Ile_{105}/Null type, while the combination of Non-null type/Val_{105}/Val_{105}/Non-Null type was not observed. We found that the genotype distributions of three GST isoenzymes in the Koreans are similar to those reported in Asians and previously reported Koreans. We believe our results, which are represented by a large population, are reliable estimates of the frequencies of the polymorphic *GST* alleles in the Koreans and will help future researches on *GST* polymorphisms.

Key Words : *Glutathione Transferase; glutathione S-transferase pi; glutathione transferase TI-1, human; GST; Polymorphism, Genetic; Korea*

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Glutathione s transferases (GSTs) consist of a superfamily of dimeric phase II metabolic enzymes that catalyse the conjugation of reduced glutathione with various electrophilic compounds (1). The human *GST* genes are divided into four major subfamilies designated as *GST*α or A, *GST*μ or *M*, *GST* θ or *T*, and *GST*π or *P* (2). The class π*GST* gene exists as a single functional gene in human, whereas class α, μ, and θ families contain multiple distinct genes, sharing ~55, 65, and 50% identity, respectively (3). Two of these subfamilies, *GSTM1* and *GSTT1*, show deletion polymorphism (4), and the *GSTP1* gene has polymorphism loci within its coding region, of which well-known are an A- to -G transition at nucleotide position 1,578 causing an isoleucine-to-valine substitution at codon 105 ($Ile^{105}Val$) in exon 5, a C- to -T base change at position 2,293 giving rise to the replacement of alanine to valine at the amino acid position 114 ($Ala^{114}Val$) in exon 6 (5, 6).

Human cytosolic GSTs have been well characterized and known to be polymorphic, with different polymorphism frequencies by ethnicity. The percentage of individuals who do not express the GSTM1 enzyme due to a homozygous gene deletion is higher in Caucasians and Asians than in Africans (7, 8). About 60% of Asians, 40% of Africans and 20% of Caucasians do not express the GSTT1 enzyme (9). These homozygous gene deletions, called null genotypes, are denoted as *GSTM1*0/*0* and *GSTT1*0/*0*. Polymorphisms of *GSTM 1, GSTT1*, and *GSTP1* have been shown to be associated with susceptibility to various forms of cancer, particular those caused by cigarette smoking (9), resistance to chemotherapy treatment (3), and disease outcomes (10). We analyzed the frequencies of the major polymorphisms of *GSTM1, GSTT1* and *GSTP1* in a Korean male population to provide a basic database for future clinical and genetic studies concerning variability in the response and/or toxicity to drugs known to be substrates for GSTs.

The study subjects are healthy individuals recruited from the health promotion center, Samsung Medical Center without any pathology. A total of 1,051 unrelated male Korean subjects (mean age, 50.7 yr; range, 35-76 yr) participated in this study. Deletion status of *GSTM1* and *GSTT1* was simultaneously determined by a multiplex polymerase chain reaction method (11). *GSTM1* and *GSTT1* genes were amplified using the following primers: 5′ GAA CTC CCT GAA AAG CTA AAG C 3′ and 5′ GTT GGG CTC AAA TAT ACG GTG G 3′ for *GSTM1* and 5′ TTC CTT ACT GGT CCT CAC ATC TC 3′ and 5′ TCA CCG GAT CAT GGC CAG CA 3′ for *GSTT1*. As an internal control, exon 7 of the *CYP1 A1* gene was co-amplified using the primers 5′ GAA CTG CCA GGC CAG CA 3′ and 5′ CAG CTG CAT TTG GAA GTG CTC 3′ . Agarose gel electrophoresis (1%) resolved amplified DNA fragments of 480, 312, and 215 bp for *GSTT1, CYP1A1* and *GSTM1*, respectively. To determine the genotypes at codon 105 and 114, respectively, the exon 5 and exon 6 of the *GSTP1* gene were amplified using the following primers: 5′ TGT GTG GCA GTC TCT CAT CC 3′ and 5′ GAA GCC CCT TTC TTG TTC A 3′ for the exon 5 and 5′ G-

CAAGCAGAGGAGAA TCT GG 3′ and 5′ CTA AGC C CA TCC CCT AGG TC 3′ for the exon 6, and directly sequenced on ABI Prism 3,700 Genetic analyzer (Applied Biosystems, Foster City, CA, U.S.A.) using the ABI Prism BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems).

The null *GSTM1* and *GSTT1* genotypes were found in 53.8% and 54.3% of the individuals, respectively. Twenty-nine percent had the null genotype for both genes. The only genetic polymorphism in *GSTP1* was $Ile^{105}Val$ in exon 5. The genotype distribution of this locus was Ile_{105}/Ile_{105} in 68.4%, Ile_{105}/Val_{105} in 29.1%, and Val_{105}/Val_{105} in 2.5%, which is in Hardy-Weinberg equilibrium by χ^2 test. The allele frequency of Ile at codon 105 was 0.83. We examined the distribution and frequencies of the combined genotypes of *GSTM1*, *GSTP 1* and *GSTM1*. For genotype combination analysis, there were 1,021 samples available where the genotyping was successful for each of three *GST* genes. Eleven out of 12 possible combinations were observed (Table 1). Five genotype combinations showed frequencies greater than 10%. The most frequently observed combination was Null type/Ile_{105}/Ile_{105}/Null type, while Non-null type/Val_{105}/Val_{105}/Non-Null type was not observed.

Polymorphisms in *GST* genes can affect the expression levels of the GST enzymes. Since GST enzymes play a vital role in cellular defense against environmentally toxic compounds, such as carcinogens, polymorphisms of *GST* gene can increase subsceptibility to diseases caused by such xenobiotics. We observed 53.8% of the Korean population were homozygous for the *GSTM1* deletion. This frequency is similar to that reported in a previous study that analyzed the *GSTM1* polymorphism in Koreans (12, 13) and also to those reported in other studies in Caucasians and Japanese (Table 2) (14, 15). We observed 54.3% of these Koreans were homozygous for the *GSTT1* deletion. This frequency is similar to that reported in other studies that analyzed the *GSTT1* polymorphism in Koreans and Japanese (12, 15), however higher than that observed in Caucasian population (Table 2) (7, 14, 16). The frequency of double nulls observed in the present study (29.1 %) is higher than that observed in a Caucasian population (14), South Indians, and Afro-Americans (17). No frequency data are available for double nulls in other Asians including Japanese and Chinese, and Koreans. Three different *GSTP1* alleles, *GSTP 1a*, *GSTP1b*, and *GSTP1c*, have been described (6). *GSTP1b* differs from *GSTP1a* by having an A→G transition at nucleotide +313, changing codon 105 from ATG (Ile) to GTC (Val). *GSTP1c* is characterized by two nucleotide transitions, A→G at +313, the same as observed in *GSTP 1b*, and C→T at +341, changing codon 114 from GCG (Ala) to GTG (Val) (5). In this study, we observed 68.4% had the Ile_{105}/Ile_{105} genotype, 29.1% had the Ile_{105}/Val_{105} genotype, and 2.5% had the Val_{105}/Val_{105} genotype, with an allelic frequency 0.83 for the Ile allele. The polymorphism GCG (Ala) →GTG (Val) at codon 114 was not observed. These results are similar to those reported in other studies in Koreans (18) and other Asians (19, 20). The Val_{105}/Val_{105} genotype was more frequent in Caucasians than in Asians (Table 3) (21). Little has been known about the combined effect of the *GSTM1*, *GSTT1*, and *GSTP1* genotypes. For all three polymorphisms, previous studies reported association with various diseases, however subsequent studies validating these findings are lacking. Recent reports and meta-analysis show that single *GST* gene polymorphisms do not significantly increase risks to various diseases (22-24), suggesting investigations on combined

Table 1. Frequency distribution of the combined genotypes for the *GSTM1*, *GSTT1* and *GSTP1* polymorphism

Combination (*GSTM1*/*GSTP1*/*GSTT1*)	Frequency % (No. of individuals)
Non-null type/Ile_{105}/Ile_{105}/Non-null type	14.89 (152)
Non-null type/Ile_{105}/Ile_{105}/Null type	18.02 (184)
Non-null type/Ile_{105}/Val_{105}/Non-null type	6.07 (62)
Non-null type/Ile_{105}/Val_{105}/Null type	6.56 (67)
Non-null type/Val_{105}/Val_{105}/Null type	1 (1)
Null type/Ile_{105}/Ile_{105}/Null type	18.71 (191)
Null type/Ile_{105}/Ile_{105}/Non-null type	16.65 (170)
Null type/Ile_{105}/Val_{105}/Non-null type	7.93 (81)
Null type/Ile_{105}/Val_{105}/Null type	10.19 (104)
Null type/Val_{105}/Val_{105}/Non-null type	0.29 (3)
Null type/Val_{105}/Val_{105}/Null type	0.59 (6)
Total	100 (1,021)

Table 2. Frequencies of the homozygous deletions at *GSTM1*, *GSTT1* loci and their combination in present study, in comparison with those on other studies in Koreans and other populations

GST genotype	Frequency, % (95% confidence intervals)				
	Choi et al. (12) n=177	Jang et al. (13) n=243	Caucasians (14) n=213	Japanese (15) n=150	The present study n=1,037
*GSTM1*0/*0*	53.7 (46.3-61.1)	55.6 (49.3-61.9)	53.5 (46.8-60.2)	51.3 (43.3-59.3)	53.8 (50.8-56.9)
*GSTT1*0/*0*	53.1 (45.7-60.5)	55.1 (48.9-61.4)	14.7 (10.2-19.8)	54.0 (46.2-62.0)	54.3 (51.3-57.4)
Combined	Not reported	Not reported	7.5 (4.4-11.9)	Not reported	29.1 (26.4-31.9)

Table 3. Frequencies of *GSTP1* polymorphisms in the present study, in comparison those in other studies on Koreans and other populations

Population	% Frequency distribution			Ile allele frequency
	Ile_{105}/Ile_{105}	Ile_{105}/Val_{105}	Val_{105}/Val_{105}	
Yim et al. (18) (n=94)	61	37	2	0.79
Jee et al. (29) (n=707)	66.8	29.3	3.9	0.81
Caucasians (21) (n=622)	47.9	40.8	11.3	0.68
Japanese (20) (n=257)	71.6	25.3	3.1	0.84
Chinese (19) (n=119)	70.6	28.6	0.8	0.71
The present study (n=1,030)	68.4	29.1	2.5	0.83

genotypes of *GSTM1*, *GSTT1* and *GSTP1*, or even in relation to other metabolizing enzymes are needed. Several studies have reported a relationship between combination of the *GST* genotype and risk of various diseases such as chronic lymphocytic leukemia, thyroid cancer and breast cancer (25-27) and some of them suggested a possible synergistic effect between *GST* genotypes (25, 27). In present study concerning combination of the *GSTM1*, *GSTP1* and *GSTT1* genotypes, noteworthy points are lack of Non-null type/Val_{105}/Val_{105}/Non-Null type and presence of Null type/Val_{105}/Val_{105}/Null type. Although finding might need further confirmation in other healthy populations, it suggests a potential difference in genetic susceptibility to various diseases in Korean population. Although our study included only male subjects, given that the genotype frequencies are not affected by sex in general (28), our data can represent the population genotype frequencies. Indeed, our data did not show any significant differences when compared with other studies that included female subjects (13, 28).

In conclusion, genotype data for polymorphic variants of *GST* genes provide further evidence for ethnic variations in metabolism and disposition. The notable merit of this study is that we genotyped all three major GST enzymes, GSTM1, GSTP1, and GSTT1, in the largest population studied, reporting the frequency distribution of the combined genotypes. We believe these data will help genetic studies on *GSTM1*, *GSTT1* and *GSTP1* polymorphisms in association with disease risks and drug effects in Koreans.

REFERENCES

1. Mannervik B. *The isoenzymes of glutathione transferase. Adv Enzymol Relat Areas Mol Biol 1985; 57: 357-417.*
2. Mannervik B, Awasthi YC, Board PG, Hayes JD, Di Ilio C, Ketterer B, Listowsky I, Morgenstern R, Muramatsu M, Pearson WR. *Nomenclature for human glutathione transferases. Biochem J 1992; 282: 305-6.*
3. Hayes JD, Pulford DJ. *The glutathione S-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. Crit Rev Biochem Mol Biol 1995; 30: 445-600.*
4. Nelson HH, Wiencke JK, Christiani DC, Cheng TJ, Zuo ZF, Schwartz BS. *Ethnic differences in the prevalence of the homozygous deleted genotype of glutathione S-transferase theta. Carcinogenesis 1995; 16: 1243-5.*
5. 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: 10004-12.*
6. 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: 641-4.*
7. Bailey LR, Roodi N, Verrier CS, Yee CJ, Dupont WD, Parl FF. *Breast cancer and CYP1A1, GSTM1, and GSTT1 polymorphism: evidence of a lack of association in Caucasians and African Americans. Cancer Res 1998; 58: 65-70.*
8. Roth MJ, Dawsey SM, Wang G, Tangrea JA, Zhou B, Ratnasinghe D, Woodson KG, Olivero OA, Poierier MC, Frye BL, Taylor PR, Weston A. *Association between GSTM1*0 and squamous dysplasia of the esophagus in the high risk region of Linxian, China. Cancer Lett 2000; 156: 73-81.*
9. Strange RC, Fryer AA. *The glutathione S-transferase: influence of polymorphism on cancer susceptibility. In: Vineis P (ed) Metabolic polymorphism and susceptibility to cancer. IARC Scientific Publication, Lyon France 1999; 231-49.*
10. Lear JY, Heagerty AH, Smith A, Bowers B, Payne CR, Smith CA, Jones PW, Gilford J, Yengi L, Alldersea J, Fryer AA, Strange RC. *Multiple cutaneous basal cell carcinomas: glutathione S-transferase (GSTM1, GSTT1) and cytochrome P450 (CYP2D6, CYP1A1) polymorphisms influence tumor numbers and accrual. Carcinogenesis 1996; 12: 1891-6.*
11. Abdel-Rahman SZ, El-Zein RA, Anwar WA, Au WW. *A multiplex PCR procedure for polymorphic analysis of GSTM1 and GSTT1 genes in population studies. Cancer Lett 1996; 107: 229-33.*
12. Choi SC, Yun KJ, Kim TH, Kim HJ, Park SG, Oh GJ, Chae SC, Nah YH, Kim JJ, Chung HT. *Prognostic potential of glutathione S-transferase M1 and T1 null genotypes for gastric cancer progression. Cancer Letters 2003; 195: 169-75.*
13. Jang SS, Jung CY, Lee SY, Lee JH, Jeon HS, Park SH, Son JW, Lee EB, Kim CH, Kam S, Park RW, Kim IS, Jung TH, Park JY. *The GST1 genotypes as a marker for susceptibility to lung cancer in Korean female never-smokers. Tuberc Respir Dis 2003; 54: 485-94.*
14. Chen CL, Liu Q, Relling MV. *Simultaneous characterization of glutathione S-transferase M1 and T1 polymorphisms by polymerase chain reaction in American whites and blacks. Pharmacogenetics 1996; 6: 187-91.*
15. Naoe T, Takeyama K, Yokozawa T, Kiyoi H, Seto M, Uike N, Ino T, Utsunomiya A, Maruta A, Jin-nai I, Kamada N, Kubota Y, Nakamura H, Shimazaki C, Horiike S, Kodera Y, Saito H, Ueda R, Wiemels J, Ohno R. *Analysis of genetic polymorphism in NQ01, GSTM1, GSTT1, and CYP3A4 in 469 Japanese patients with therapy-related leukemic/myelodysplastic syndrome and de novo acute myeloid leukemia. Clin Cancer Res 2000; 6: 4091-5.*
16. Zhang H, Ahmadi A, Arbman G, Zdolsek J, Carstensen J, Nordenskjold B, Soderkvist P, Sun XF. *Glutathione S- transferase T1 and M1 genotypes in normal mucosa, transitional mucosa and colorectal adenocarcinoma. Int J Cancer 1999; 84: 135-8.*
17. Naveen AT, Adithan C, Padmaja N, Shashindran CH, Abraham BK, Satyanarayanamoorthy K, Anitha P, Gerard N, Krishnamoorthy R. *Glutathione S-transferase M1 and T1 null genotype distribution in south Indians. Eur J Clin Pharmacol 2004; 60: 403-6.*
18. Yim JJ, Yoo CG, Lee CT, Kim YW, Han SK, Shim YS. *Lack of association between glutathione S-transferase P1 polymorphism and COPD in Koreans. Lung 2002; 180: 119-25.*
19. Wang J, Deng Y, Cheng J, Ding J, Tokudome S. *GST genetic poly-*

morphisms and lung adenocarcinoma susceptibility in a Chinese population. Cancer Lett 2003; 201: 185-93.

20. Kihara M, Noda K. *Lung cancer risk of the GSTM1 null genotype is enhanced in the presence of the GSTP1 mutated genotype in male Japanese smokers. Cancer Lett 1999; 137: 53-60.*
21. Schneider J, Bernges U, Philipp M, Woitowitz HJ. *GSTM1, GSTT1, and GSTP1 polymorphism and lung cancer risk in relation to tobacco smoking. Cancer Lett 2004; 208: 65-74.*
22. Ntais C, Polycarpou A, Ioannidis JP. *Association of GSTM1, GSTT1, and GSTP1 gene polymorphisms with the risk of prostate cancer: a meta-analysis. Cancer Epidemiol Biomarkers Prev 2005; 14: 176-81.*
23. Vogl FD, Taioli E, Maugard C, Zheng W, Pinto LF, Ambrosone C, Parl FF, Nedelcheva-Kristensen V, Rebbeck TR, Brennan P, Boffetta P. *Glutathione S-transferases M1, T1, and P1 and breast cancer: a pooled analysis. Cancer Epidemiol Biomarkers Prev 2004; 13: 1473-9.*
24. Smits KM, Gaspari L, Weijenberg MP, Dolzan V, Golka K, Roemer HC, Nedelcheva Kristensen V, Lechner MC, Mehling GI, Seidegard J, Strange RC, Taioli E. *Interaction between smoking, GSTM1 deletion and colorectal cancer: results from the GSEC study. Biomarkers 2003; 8: 299-310.*
25. Martin Y, Alison C, Chantelle H, Zsofia KJ, Elanie S, Rosalind E, Estella M, Daniel C, Richard H. *Relationship between glutathione S-transferase M1, T1, and P1 polymorphism and chronic lymphocytic leukemia. Blood 2002; 99: 4216-8.*
26. Kathleen ME, Qiuyin C, Shu XO, Fan J, Zhu TL, Qi D, Gao YT, Wei Z. *Genetic polymorphism in GSTM1, GSTP1, and GSTT1 and the risk for breast cancer: results from the Shanghai breast cancer study and meta-analysis. Cancer Epidemiol Biomarkers Prev 2004; 13: 197-204.*
27. Jorge G, Sonia R, Octavia MG, Lsabel M, Teresa CF, Edward L, Luzia G, Julieta EP, Jose R. *Combined effects of glutathione S-transferase polymorphisms and thyroid cancer risk. Cancer Genet Cytogenet 2004; 151: 60-7.*
28. Garte S, Gspari L, Alexandrie AK, Ambrosone C, Autrup H, Autrup JL. *Metabolic gene polymorphism frequencies in control populations. Cancer Epidemiol Biomarkers Prev 2001; 10: 1239-48.*
29. Jee SH, Lee JE, Kim S, Kim JH, Um SJ, Lee SJ, Namkoong SE, Park JS. *GSTP1 polymorphism, cigarette smoking and cervical cancer risk in Korean women. Yonsei Med J 2002; 43: 712-6.*