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 GSTT1 null genotype. The genotypic distribution of GSTP1 was Ile<sup>105</sup>/Ile<sup>105</sup> in 68.4%, Ile<sup>105</sup>/Val<sup>105</sup> in 29.1%, and Val<sup>105</sup>/Val<sup>105</sup> in 2.5%. The most frequently observed combination of GSTM1, GSTP1 and GSTT1 genotypes was Null type/Ile<sup>105</sup>/Ile<sup>105</sup>/Null type, while the combination of Non-null type/Val<sup>105</sup>/Val<sup>105</sup>/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

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 gene families are divided into four major subfamilies designated as GST<sub>α</sub>, GST<sub>μ</sub>, GST<sub>π</sub> or GST<sub>θ</sub> or T, and GST<sub>π</sub> or P (2). The class θ 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<sup>105</sup>/Val<sup>105</sup>) 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<sup>114</sup>/Val<sup>114</sup>) 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<sup>0</sup>/0<sup>0</sup> and GSTT1<sup>0</sup>/0<sup>0</sup>. Polymorphisms of GSTM1, 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 CCT CCT GAA AAG CTA AAG C 3′ and 5′ GTT GGG CTC AAA TAT ACG GTG G 3′ for GSTM1 and 5′ TTG 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 CYP1A1 gene was co-amplified using the primers 5′ GAA CTG CCA GGC CAG CA 3′ and 5′ CAG CTC CAT TTG GAA GTG CTC 3′. Aagarose 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 TTC CAT CC 3′ and 5′ GAA GCC CCT TTC TTG TTA 3′ for the exon 5 and 5′ G-
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 \( \chi^2 \) test. The allele frequency of Ile at codon 105 was 0.83. We examined the distribution and frequencies of the combined genotypes of GSTM1, GSTP1 and GSTT1. 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). Table 2 shows 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 susceptibility 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 on Koreans (14) and other populations (19–21). 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 (12, 13), however higher than that observed in Caucasian population (Table 2). 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, Chinese, and Koreans. Three different GSTP1 alleles, GSTP1a, GSTP1b, and GSTP1c, have been described (6). GSTP1b differs from GSTP1a by having an \( A \rightarrow G \) transition at nucleotide +313, changing codon 105 from ATG (Ile) to GTC (Val). GSTP1c is characterized by two nucleotide transitions, \( A \rightarrow G \) at +313, the same as observed in GSTP1b, and \( C \rightarrow T \) at +341, changing codon 114 from GCG (Ala) to GTG (Val). 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) \( \rightarrow GTG \) (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/GSTT1/GSTP1) | 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_/Non-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_/Ile^105_/Null type | 7.93 (81) |
| Null type/Ile^105_/Val^105_/Null type | 10.19 (104) |
| Null type/Ile^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 (16) | n=1,037 |
| GSTM1*0/*0 | 53.7 (46.3-61.1) |
| GSTT1*0/*0 | 53.1 (45.7-60.5) |
| Combined | 53.8 (50.8-56.9) |

### 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 |
|------------|--------------------------|---------------------|
| Yim et al. (18) (n=94) | Ile^105_/Ile^105_/ | 61 |
| Jee et al. (29) (n=707) | Val^105_/Val^105_/ | 29.3 |
| Caucasians (21) | Ile^105_/ | 47.9 |
| Japanese (20) | Val^105_/ | 71.6 |
| Chinese (19) | Ile^105_/ | 71.6 |
| The present study (n=1,030) | Val^105_/ | 68.4 |

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).
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/Val105/Val105/Non-Null type and presence of Null type/Val105/Val105/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.

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