Gene Frequencies of the Polymorphic Human Glutathione S-transferase Class Pi: are they Race-dependent?

Sasichai Kangsadalampai, Pornpen Gamnarai and Panadda Rojpibulstit
Division of Biochemistry, Faculty of Medicine,
Thammasat University, Rangsit Campus, Klong-Luang,
Patum Thani, 12121, Thailand

Abstract

In this study, gene and genotype frequencies of glutathione-S-transferase class pi (GSTP) (Ile105Val and Ala114Val) were determined in Thais (n=100). The study showed that the frequencies of Ile105 and Val105 alleles were 0.84 and 0.16 while those of Ala114 and Val114 were 0.98 and 0.02, respectively. In addition, the distribution of the genotype frequencies at the codons 105 and 114 were in good agreement with those expected in a Hardy-Weinberg equilibrium. However, the genotype frequency of the heterozygous Ile114Val was very low, and the homozygous Val114 was not found in the group of Thai individuals tested as previously reported in other population. It is important to note that the frequencies of both Val105 and Val114 variant alleles from Thais and other Asians are lower than those from Caucasians. This indicates that a distribution of the GSTP gene frequencies may depend upon the racial groups as observed in many other genes.

Keywords : Glutathione-S-transferase, polymorphism, gene and genotype frequencies

1. Introduction

Human glutathione S-transferases (GSTs: EC 2.5.1.18) play an important role in phase II detoxification process [1]. The enzymes catalyse a conjugation reaction between glutathione (GSH) and a number of toxic substances to reduce their toxicity [2]. The enzymes can be divided into 6 major classes; alpha (GSTA), mu (GSTM), pi (GSTP), theta (GSTT), sigma (GSTS) and omega (GSTO) [more detail see ref. 3].

The pi class GST has been well-known for its high degree of polymorphism. Initially, allelic variants, GSTP1*A, GSTP1*B, GSTP1*C and GSTP1*D were detected [4,5] and physiologically important differences between these variants were reported [6,7]. It was subsequently clearly demonstrated that these four alleles are the products of amino acid substitutions in codons 105 (exon 5) and 114 (exon 6): GSTP1*A (Ile105:Ala114), GSTP1*B (Val105:Ala114), GSTP1*C (Val105:Val114), GSTP1*D (Ile105:Val114). This revealed that Val105 variants (GSTP1*B and *C) exhibit high K_m for 1-chloro-2,4-dinitrobenzene (CDNB) and high V_{max} for carcinogenic epoxide of benzo(a)pyrene (BPDE) [6,7]. In addition, many previous studies reported the association between the GSTP polymorphism and some cancers [e.g., ref. 8,9], as well as some non-cancerous diseases [e.g., ref. 10,11].

Since the polymorphism in the GSTP possibly contributes to the altered level of drug or carcinogen detoxification and is likely to involve susceptibility to many diseases, it was the aim of this work to determine the distribution of gene and genotype frequencies in Thais for further study on their roles in individual susceptibility to diseases in Thai population.

2. Materials and Methods

Specimen collection

Blood samples were obtained from 100 Thai blood donors who live in the central region of Thailand. The donors were comprised of 50 males and 50 females with an averaged age of 20.29 ± 1.78 years over the range of 18 to 26.

DNA amplification and genotyping

DNA was isolated from donor's leucocytes by the QIAamp DNA extraction kit (Qiagen, USA) according to the manufacturer's recommendation. The polymerase chain reaction was used to amplify a 192 bp and 216 bp DNA fragments encompassing codon 105 of exon 5 and codon 114 of exon 6 of the *GSTP* gene, respectively. A pair of each exon specific primers (exon 5: 5' CTCTATGGGAAGGACC AGCAGGAG 3' and 5' CAAGCCACCTGAGG GGTAAGG 3'; exon 6: 5' GTTGTGGGGAGC AAGCAGAGG 3' and 5' CACAATGAAGGTC TTGCCTCCC 3') were used in a polymerase chain reaction that was performed as described previously [10]. In brief, the exons 5 and 6 of the *GSTP* gene were amplified in a final volume of 50 µl reaction that consists of 50-100 ng DNA template, PCR buffer (50 mM KCl, 20 mM Tris-HCl pH 8.0, 0.05% Tween 20) 1.5 mM $MgCl_2$, 60 µM dNTPs, 0.2 µM each primer and 1.25 units *Taq* DNA polymerase (Promega, USA). The PCR condition was 95°C; 40 sec, 65°C; 40 sec, 72°C; 40 sec (40 cycles). The PCR products of exons 5 and 6 were separately genotyped by *Bsm*AI and *Aci*I digestion (New England Biolabs, USA), respectively. The digested fragments were separated by 3% MetaPhor agarose (Cambrex, USA) gel electrophoresis in TBE buffer (0.089 M Tris base, 0.089 M boric acid, 0.002 M EDTA, pH 8.0) and stained with 0.5 µg/ml ethidium bromide.

Chi-square testing was used to test the significant difference of the GSTP genotype frequencies between observed and expected values. One-way analysis of variance (ANOVA) using SPSS version 13.0 (SPSS, Cary, USA) was applied to evaluate the difference of the gene frequencies between races.

3. Results

DNA amplification and genotyping of *GSTP* gene

DNA amplification at a specific region in *GSTP* gene and a suitable restriction endonuclease digestion were applied to do genotyping of the *GSTP* gene in 100 Thai individuals. The amplified product of exon 5 was 192 bp. The Ile105Val substitution in this exon created a *Bsm*AI cleavage site. Therefore, after the *Bsm*A1 digestion of the 192 bp amplified exon 5 fragment (figure 1A), the PCR product of Ile105 homozygote was uncut. In contrast, the 192 bp fragment of Val105 homozygote was cleaved into 83 and 109 bp fragments. In the case of a heterozygote, half of the amplified product was cleaved into 83 and 109 bp fragments while another half remained undigested. For the polymorphism in exon 6, the amplified product of this exon was 216 bp and the Ala114Val replacement deleted a *Aci*I digestion site. The 216 bp amplified DNA of the Ala114 homozygote can be cut by *Aci*I into 92 and 124 bp fragments while that of Val114 homozygote was undigested (figure 1B). A sample of the Ala114Val heterozygote showed a combination of both patterns, i.e., an undigested (216 bp) and two digested (92 and 124 bp) fragments.

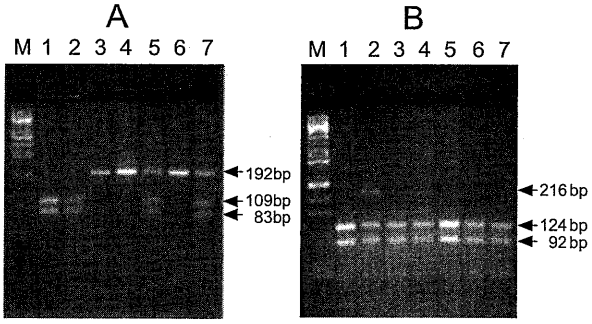

Figure 1. Genotyping of exons 5 and 6 of *GSTP* gene. The 100 bp DNA marker was run in lane M.
A. PCR-RFLP of the Ile105Val substitution. Lanes 1 and 2 = homozygous Val105; lanes 3, 4 and 6 = homozygous Ile105; lanes 5 and 7 = heterozygous Ile105Val.
B. PCR-RFLP of the Ala114Val substitution. Lanes 1, 3 to 7 = homozygous Ala114; lane 2 = heterozygous Ala114Val.

Distribution of GSTP genotypes in Thai population

To verify whether the Val105 and Val114 variant alleles were distributed in equilibrium in Thai population as reported in other racial groups worldwide (especially in Caucasians), the PCR-RFLP method was used to determine the frequencies of these alleles in samples of 100 Thai people. Table 1 shows that the frequencies of the variant alleles at codons 105 and 114 in Thais were 0.16 and 0.02,

respectively, indicating that the frequency of the former allele was more common than that of the latter. The observed and expected genotype frequencies of the polymorphisms in exons 5 and 6 were calculated and revealed that the distribution of codon 105 genotypes in this population was not significantly different from that expected in a Hardy-Weinberg equilibrium. Although there was no Val114/Val114 genotype among 100 samples tested, the distribution of codon 114 genotypes can also be determined. The data demonstrated that the distribution of the genotypes at codon 114 was also in agreement with that expected in the Hardy-Weinberg equilibrium.

Table 1. Genotype and gene frequencies of the GSTP Ile105Val and Ala114Val polymorphisms in the Thai population (n=100)

		Genotype (%)			Gene frequency	
Exon 5		Ile105/Ile105	Ile105/Val105	Val105/Val105	Ile105	Val105
	Observed	70	28	2	0.84	0.16
	Expected	70.56	26.88	2.56		
				$\chi^2 = 0.17$; $p < 0.95$; not significant		
Exon 6		Ala114/Ala114	Ala114/Val114	Val114/Val114	Ala114	Val114
	Observed	96	4	0	0.98	0.02
	Expected	96.04	3.92	0.004		
				$\chi^2 = 0.04$; $p < 0.99$; not significant		

4. Discussion

Polymorphisms in *GSTP* gene have been reported in a number of racial groups world-wide and the data revealed that races appeared to affect the distribution of the gene frequencies [12]. One-way analysis of variance (ANOVA) of the gene frequencies in Table 2 shows that the gene frequencies of both Val105 and Val114 alleles are significantly low in the Asians compared to the Caucasians. This was confirmed by the frequencies from Thais in this study. Therefore, it was speculated that the presence of both Val105 and Val114 alleles in the Caucasian population would arise before the divergence of the major racial groups and appeared to be stable over a considerable time. On the other hand, at the same period of time, these variant alleles in the Asians would not occur as frequently as in the Caucasians and remained constant thereafter. In addition, regarding to the frequencies of the Val105 and Val114 variant alleles, the Aborigines and the Asians may have the same descendant while the Caucasians probably originated from the Africans. Theoretically, factors influencing the genetic diversity include natural selection, gene flow between continents, founder effect and genetic drift. In this study, the first three factors seem to have more effect on the frequencies of the *GSTP* alleles between the Caucasians and the Asians than the genetic drift as the latter factor is likely to cause a change between generations rather than between races.

Table 2. Gene frequencies of GSTP Ile105Val and Ala114Val in some different populations

Population	Codon 105		Codon 114		Ref.
	Ile	Val	Ala	Val	
Australian*	0.66	0.34	0.93	0.07	12
Estonian*	0.65	0.35	0.89	0.11	13
Finish*	0.72	0.28	0.91	0.09	14
German*	0.67	0.33	0.88	0.12	15
Chinese*	0.81	0.19	0.99	0.01	12
Korean*	0.82	0.18	-	-	16
Thai*	0.84	0.16	0.98	0.02	This study
Aborigines#	0.89	0.11	1.00	0	12
African‡	0.58	0.42	0.90	0.10	5

* $p = 0.001$ (codon 105) and $p = 0.008$ (codon 114), significantly different between the Caucasians and the Asians

\# $p = 0.063$ (codon 105) and $p = 0.333$ (codon 114), not significantly different between the Aborigines and the Asians

‡ $p = 0.072$ (codon 105) and $p = 0.929$ (codon 114), not significantly different between the Africans and the Caucasians

Analysis of the previously reported data on association between GSTP Ile105Val genotypes and clinical pathology of many organ systems are inconclusive. For examples, Wang *et al.*[17] demonstrated that the Val105 allele associated with lung cancer but To-Figueras *et al.* [18] showed the opposite result. This kind of debate was also found in breast cancer [19,20] and some other diseases. This implied that, besides

the GSTP genotypes, there could be other factors such as genotypes of other enzymes, foods, drugs, and smoking etc. participating in diseases.

Although the roles of GSTP genotypes on clinical outcomes are still controversial, the gene and genotype frequencies of GSTP may be useful in the field of pharmacogenomics which is going to become more important in modern medicine in selecting an appropriate drug and its dosage for each patient. The gene frequencies of Val105 and Val114 from Thais are similar to those from Asian populations but lower than those in the Caucasians. This result is similar to many recent studies on gene frequencies of other enzymes, which suggested the significant differences in the gene frequencies between major racial groups, for examples, GSTP omega class [21], cytochrome P450 [22], arylamine N-acethyl transferase [23] and the blood coagulation factor XIII A subunit [24]. This suggests that data of gene or genotype frequency from one racial group could not immediately be applied to others. Therefore, determination of the gene and genotype frequencies in populations of different races is necessary.

In conclusion, gene and genotype frequencies of the GSTP from Thai population studies have been carried out using the PCR-RFLP based technique. The result demonstrated a difference in the gene frequencies between those from the Asians and those from the Caucasians. The data confirmed the difference in the gene frequencies between major racial groups.

5. Acknowledgements

Special appreciation is given to all blood donors whose DNA samples were used in this study. The work was supported by a grant from the Faculty of Medicine, Thammasat University.

6. References

[1] Hayes, J.D. and Pulford, D.J., 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., Vol. 30, pp. 445-600, 1995.

[2] Armstrong, R.N., Structure, Catalytic Mechanism, and Evolution of the Glutathione Transferases, Chem. Res. Toxicol., Vol. 10, pp. 2-18, 1997.

[3] Hayes, J.D., Flanagan, J.U. and Jowsey, I.R., Glutathione Transferases, Annu. Rev. Pharmacol. Toxicol., Vol. 45, pp. 51-88, 2005.

[4] Ali-Osman, F., Akande, O., Antoun, G., Mao, J.X. and 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., Vol. 272, 10004-10012, 1997.

[5] Watson, M.A., Stewart, R.K., Smith, G.B., Massey, T.E. and Bell, D.A., Human Glutathione S-transferase P1 Polymorphisms: Relationship to Lung Tissue Enzyme Activity and Population Frequency Distribution, Carcinogenesis, Vol. 19, pp. 275-280, 1998.

[6] Hu, X., O'Donnell, R., Srivastava, S.K., Xia, H., Zimniak, P., Nanduri, B., Bleicher, R.J., Awasthi, S., Awasthi, Y.C., Ji, X. and Singh, S.V., Active Site Architecture of Polymorphic forms of Human Glutathione S-transferase P1-1 Accounts for their Enantioselectivity and Disparate Activity in the Glutathione Conjugation of 7β, 8α-Dihydroxy-9α, 10α-oxy-7,8,9,10-Tetrahydrobenzo(a)pyrene, Biochem. Biophys. Res. Commun., Vol. 235, pp. 424-428, 1997.

[7] Sundberg, K., Johansson, A.S., Stenberg, G., Widersten, M., Seidel, A., Mannervik, B. and Jernstrom, B., Differences in the Catalytic Efficiencies of Allelic Variants of Glutathione Transferase P1-1 Towards Carcinogenic Diol Epoxides of Polycyclic Aromatic Hydrocarbons, Carcinogenesis, Vol. 19, pp. 433-436, 1998.

[8] Harries, L.W., Stubbins, M.J., Forman, D., Howard, G.C. and Wolf, C.R., Identification of Genetic Polymorphisms at the Glutathione S-transferase Pi Locus and Association with Susceptibility to Bladder, Testicular and Prostate Cancer, Carcinogenesis, Vol. 18, pp. 641-644, 1997.

[9] Matthias, C., Bockmuhl, U., Jahnke, V., Harries, L.W., Wolf, C.R., Jones, P.W., Alldersea, J., Worrall, S.F., Hand, P., Fryer,

A.A. and Strange, R.C., The Glutathione S-Transferase GSTP1 Polymorphism: Effects on Susceptibility to Oral/pharyngeal and Laryngeal Carcinomas, Pharmacogenetics, Vol. 8, pp. 1-6, 1998.

[10] Menegon, A., Board, P.G., Blackburn, A.C., Mellick, G.D. and Le Couteur, D.G., Parkinson's Disease, Pesticides, and *Glutathione Transferase* Polymorphisms, Lancet, Vol. 352, pp. 1344-1346, 1998.

[11] Kelada, S.N., Stapleton, P.L., Farin, F.M., Bammler, T.K., Eaton, D.L., Smith-Weller, T., Franklin, G.M., Swanson, P.D., Longstreth, W.T. Jr. and Checkoway, H., Glutathione S-transferase M1, T1, and P1 Polymorphisms and Parkinson's Disease, Neurosci. Lett., Vol. 337, pp. 5-8, 2003.

[12] Harris, M.J., Coggan, M., Langton, L., Wilson, S.R. and Board, P.G., Polymorphism of the Pi Class Glutathione S-transferase in Normal Populations and Cancer Patients, Pharmacogenetics, Vol. 8, pp. 27-31, 1998.

[13] Juronen, E., Tasa, G., Veromann, S., Parts, L., Tiidla, A., Pulges, R., Panov, A., Soovere, L., Koka, K. and Mikelsaar, A.V., Polymorphic Glutathione S-transferases as Genetic Risk Factors for Senile Cortical Cataract in Estonians, Invest. Ophthalmol. Vis. Sci., Vol. 41, pp. 2262-2267, 2000.

[14] Saarikoski, S.T., Voho, A., Reinikainen, M., Anttila, S., Karjalainen, A., Malaveille, C., Vainio, H., Husgafvel-Pursiainen, K. and Hirvonen, A., Combined effect of Polymorphic GST Genes on Individual Susceptibility to Lung Cancer, Int. J. Cancer, Vol. 77, pp. 516-521, 1998.

[15] Stanulla, M., Schrappe, M., Brechlin, A.M., Zimmermann, M. and Welte, K., Polymorphisms Within Glutathione S-Transferase Genes (GSTM1, GSTT1, GSTP1) and Risk of Relapse in Childhood B-cell Precursor Acute Lymphoblastic Leukemia: a Case-control Study, Blood, Vol. 95, pp. 1222-1228, 2000.

[16] Pae, C.U., Kim, J.J., Lee, S.J., Lee, C.U., Lee, C., Paik, I.H., Park, H.R., Yang, S. and Serretti, A., Association Study Between Glutathione S-transferase P1 Polymorphism and Schizophrenia in the Korean Population. Prog. Neuropsychopharmacol. Biol. Psychiatry, Vol. 27, pp. 519-523, 2003.

[17] Wang, J., Deng, Y., Cheng, J., Ding, J. and Tokudome, S., GST Genetic Polymorphisms and Lung Adenocarcinoma Susceptibility in Chinese Population. Cancer Lett. Vol. 201 pp. 185-193, 2003.

[18] To-Figueras, J., Gene, M., Gomez-Catalan, J., Pique, E., Borrego, N., Caballero, M., Cruellas, F., Raya, A., Dicenta, M. and Corbella, J., Microsomal Epoxide Hydrolase and Glutathione S-transferase Polymorphisms in Relation to Laryngeal Carcinoma Risk, Cancer Lett., Vol. 187 pp. 95-101, 2002.

[19] Helzlsouer, K.J., Selmin, O., Huang, H.Y., Strickland, P.T., Hoffman, S., Alberg, A.J., Watson, M., Comstock, G.W. and Bell, D., Association Between Glutathione S-Transferase M1, P1 and T1 Genetic Polymorphisms and Development of Breast Cancer, J. Natl. Cancer Inst., Vol. 90, pp. 512-518, 1998.

[20] Woldegiorgis, S., Ahmed, R.C., Zhen, Y., Erdmann, C.A., Russell, M.L. and Goth-Goldstein, R. Genetic Polymorphism in three Glutathione S-transferase Genes and Breast Cancer risk, Lawrence Berkeley National Laboratory, University of California, Paper LBNL-50365, 2002. http://repositories.cdlib.org/lbnl/LBNL-50365

[21] Marahatta, S.B., Punyarit, P., Bhudisawasdi, V., Paupairoj, A., Wongkham, S. and Petmitr, S., Polymorphism of Glutathione S-transferase Omega Gene and Risk of Cancer, Cancer Lett, Vol. 236, pp. 276-281, 2006.

[22] Oscason, M., Ingelman-Sunberg, M., Daly, A.K. and Nebert, D.B., Human Cytochrome P450 (CYP) alleles, http://www.imm.ki.se/CYPalleles/2000.

[23] Hein, D.W., Grant, D.M. and Sim, E., Arylamine N-acetyltransferase (EC2.3.1.5), http://www.louisville.edu/medschool/pharmacology/NAT.html/2000.

[24] Okumura, T., Yamada, T. and Park, S.C., Ichinose, A., No Val34Leu Polymorphism of the Gene for Factor XIIIA Subunit was Detected by ARMS-RACE Method in three Asian Populations, J. Thromb. Haemost., Vol. 1, pp. 1856-1857, 2003.