CHAPTER V
RESULTS

1. DNA amplification and genotyping of *GSTP* gene in Thai population

Amplification and genotyping of *GSTP* gene at codon 105 (exon 5) and codon 114 (exon 6) were undertaken in Thai volunteers (n = 100). PCR product of exon 5 of *GSTP* gene was 192 bp (Figure 10A). Genotyping of codon 105 in this exon was performed by a PCR-RFLP technique. The Ile105Val substitution in this exon created a *Bsm*AI cleavage site. Therefore, after the *Bsm*AI digestion of the 192 bp amplified exon 5 fragment, the PCR product of Ile105 homozygote (192 bp) was uncut. In contrast, the 192 bp fragment of Val105 homozygote was cut into 109 and 83 bp fragments. In the case of Ile105Val heterozygote, half of the amplified product was cleaved into 83 and 109 bp fragments but another half remained undigested (Figure 10B).

For exon 6 of *GSTP* gene, the PCR product of this exon was 216 bp (Figure 11A). Genotyping of codon 114 in this exon was also done by the PCR-RFLP. The Ala114Val substitution eliminated a *Aci*I digestion site. The 216 bp PCR product of the Ala114 homozygote can be cleaved by *Aci*I into 92 and 124 bp fragments while that of Val114 homozygote was undigested (Figure 11B). Digested sample of the Ala114Val heterozygote showed three bands (216, 124 and 92 bp), indicating a mixture of cleaved and uncleaved fragments.

2. Distribution of GSTP genotypes in Thai population

The distribution of GSTP Ile105Val and Ala114Val have previously been studied in various populations (see Tables 6 and 7 in chapter III). Thus, in this study, the distribution of gene and genotype frequencies of both GSTP polymorphisms in Thais were examined. Table 15 shows that the gene frequencies of the Val105 and Val114 allelic variants were 0.16 and 0.02 in Thai population. For table 16, the observed and expected genotype frequencies of the polymorphisms in exons 5 and 6 were calculated (according to a Hardy-Weinberg Law: $p^2 + 2pq + q^2 = 1$) and found that they were not significantly different. Therefore, the results indicated that the distribution of codons 105 and 114 genotypes in Thai population were in agreement with those expected in a Hardy-Weinberg equilibrium ($\chi^2 = 0.17$; $p > 0.05$ for codon 105 and $\chi^2 = 0.04$; $p > 0.05$ for codon 114).

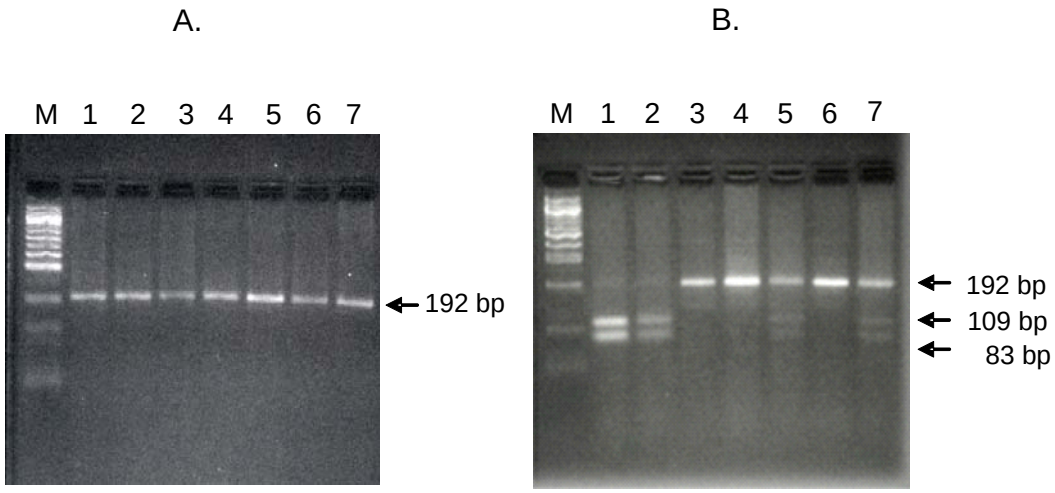

Figure 10 DNA amplification and genotyping of exon 5 of *GSTP* gene

A. Undigested 192 bp PCR products (lanes 1 to 7) and 100 bp DNA marker (lane M)

B. PCR-RFLP of the Ile105Val substitution. Lanes 1 and 2 = homozygous Val105 (the incompletely digested 192 bp fragment also appeared in lane 1); lanes 3, 4 and 6= homozygous Ile105; lanes 5 and 7 = heterozygous Ile105Val. The 100 bp marker was run in lane M.

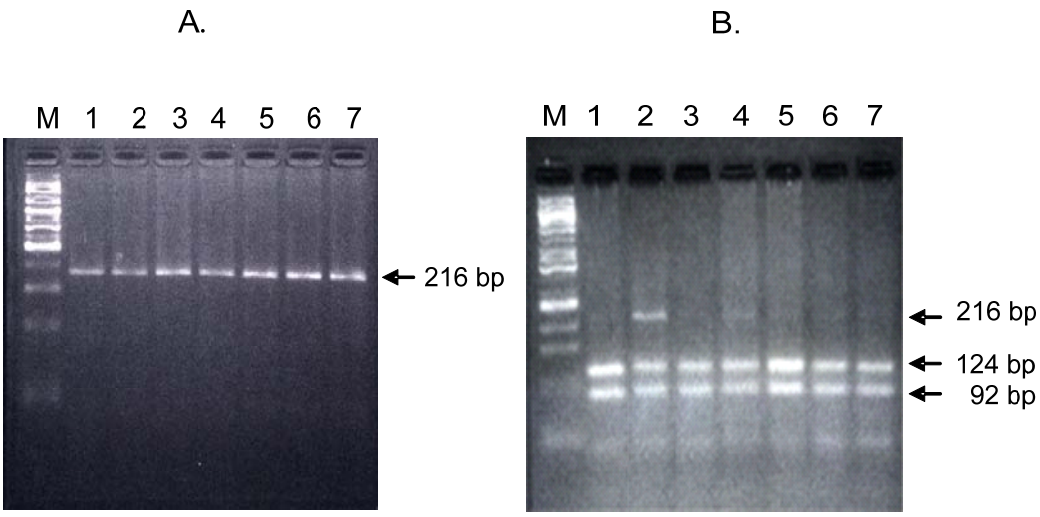

Figure 11 DNA amplification and genotyping of exon 6 of *GSTP* gene

A. Undigested 216 bp PCR products (lanes 1 to 7) and 100 bp DNA marker (lane M)

B. PCR-RFLP of the Ala114Val substitution. Lanes 1, 3 to 7 = homozygous Ala114; lane 2 = heterozygous Ala114Val (the intensity of the 124 and 92 bp fragments in this lane was rather weak). The 100 bp marker were run in lane M.

Table 15 Genotype and gene frequencies of GSTP Ile105Val and Ala114Val in Thai population (n = 100)

	Genotype (%)			**Gene frequency**	
Exon 5	Ile105/Ile105	Ile105/Val105	Val105/Val105	Ile105	Val105
	70	28	2	0.84	0.16
Exon 6	Ala114/Ala114	Ala114/Val114	Val114/Val114	Ala114	Val114
	96	4	0	0.98	0.02

Table 16 A test for genotype frequency equilibrium of GSTP Ile105Val and Ala114Val in Thai population

	Genotype	**Observed (o)**	**Expected (e)**	**(o-e) = d**	**d^2**	**d^2/e**
Ile105Val substitution	Ile/Ile	70	70.56	-0.56	0.3136	0.0044
	Ile/Val	28	26.88	1.12	1.2544	0.0467
	Val/Val	2	2.56	-0.56	0.3136	0.1225
						$\sum d^2/e = 0.1736$
	Chi-square = 0.17, df = 2, p>0.05					
Ala114Val substitution	Ala/Ala	96	96.04	-0.04	0.0016	0.0000
	Ala/Val	4	3.92	0.08	0.0064	0.0016
	Val/Val	0	0.04	-0.04	0.0016	0.0400
						$\sum d^2/e = 0.0416$
	Chi-square = 0.04, df = 2, p>0.05					

3. GSTP genotypes and dyspeptic patients

An association between GSTP genotypes and individual susceptibility to peptic ulcer was studied in 308 dyspeptic patients. Based on gastric pathology, the patients were divided into two groups: ulcer (cases, n = 107) and non-ulcer dyspepsia or NUD (controls, n = 201). The patients data were shown in Table 17.

Table 18 shows that the gene frequencies of GSTP at codons 105 and 114 from ulcerative (cases) and from non-ulcerative dyspeptic patients (controls) were not different. In contrast, the genotype frequencies of both GSTP Ile105 and Val105 homozygotes were higher in cases than those that observed in controls (66.4% vs 56.7% for Ile105 and 7.4% vs 1.5% for Val105). However, the frequency of heterozygous Ile105Val in cases was lower than that in controls (26.2% vs 41.8%). Moreover, statistical analysis using Chi-square test revealed that the frequencies of both homozygous Val105 and heterozygous Ile105Val were significantly different between case and control ($p < 0.05$) supporting the above interpretation. On the other hand, the gene frequency of Val114 in this study was very low in both cases and controls (0.01 and 0.02, respectively). In addition, there was no homozygous Val114 in both patient groups (similar to those reported in many racial groups in Table 7) implying that this rare Val114 allele could not play an important role in development of peptic ulcer. Thus, the following study would focus on the effect of GSTP genotype only at codon 105 on the susceptibility to peptic ulceration. Consequently, the patients participating in the study had to be Ala114 homozygotes.

4. Analysis of GSTP genotype frequency and gastric pathology

To study the role of GSTP polymorphism at codon 105 on gastric ulceration, both cases (ulcerative dyspeptic patients) and controls (NUD) were selected only the Ala114 homozygotes as mentioned above (see Table 19). Table 20 shows that the odds ratio for homozygous Val105 genotype was 4.290 (95% CI, 1.100-16.722, $p = 0.030$). This result indicated that the dyspeptic patients who were Ala114 homozygotes could have high risk to peptic ulcer disease when they had genotypes of homozygous Val105. However, the patients with heterozygous Ile105Val seemed to have lower risk to peptic ulcer [OR = 0.563 (95% CI, 0.333-0.952), $p = 0.031$]. Thus, the heterozygous Ile105Val genotype might be a protective factor to peptic ulceration while the homozygous Val105 genotype appeared to be risk factor. However, some reported confounded factors such as sex, age, *H. pylori* infection, could not be ruled out. The effects of these confounders were examined by a stratified analysis.

Table 17 Endoscopic findings, sex and age of dyspeptic patients in this study (n = 308)

Endoscopic findings	n	Male/Female	Age (year)	
			Range	Mean ± SD
Ulcer	107	78 / 29	16 - 88	52.09 ± 17.84
Non-ulcer dyspepsia (NUD)	201	108 / 93	17 - 90	44.12 ± 16.07

Table 18 Genotype and gene frequencies of GSTP Ile105Val and Ala114Val in case and control study (107 ulcerative patients and 201 NUD patients**)**

	Genotype			Gene frequency	
Exon 5	**Ile105/Ile105**	**Ile105/Val105**	**Val105/Val105**	**Ile105**	**Val105**
Case	71 (66.4%)	28 (26.2%)	8 (7.4%)	0.79	0.21
Control	114 (56.7%)	84 (41.8%)	3 (1.5%)	0.78	0.22
Exon 6	**Ala114/Ala114**	**Ala114/Val114**	**Val114/Val114**	**Ala114**	**Val114**
Case	105 (98.1%)	2 (1.9%)	0 (0%)	0.99	0.01
Control	194 (96.5%)	7 (3.5%)	0 (0%)	0.98	0.02

Table 19 Endoscopic findings, sex and age of dyspeptic patients (selected only Ala114 homozygotes, n = 299)

Endoscopic findings	n	Male/Female	Age (year)	
			Range	Mean ± SD
Ulcer	105	77 / 28	16 - 88	52.22 ± 17.99
Non-ulcer dyspepsia (NUD)	194	64 / 130	17 - 90	43.84 ± 16.14

Table 20 Analysis of an association between the GSTP genotype at codon 105 and a risk to peptic ulcer disease

GSTP genotype	Case (n = 105)	Control (n = 194)	Odds ratio (95%CI)	p-value
Ile105/Ile105	69	111	reference	reference
Ile105/Val105	28	80	0.563 (0.333 - 0.952)	0.031
Val105/Val105	8	3	4.290 (1.100 – 16.722)	0.030

5. Detection of *H. pylori* by PCR amplification of *hpglm*M gene

H. pylori infection was reported as one of the factors associated with peptic ulcer and was commonly found among Thai people (Perez-Perez *et al*., 1990; Pongchairerks *et al*., 1997), therefore, in this study on genotype susceptibility to peptic ulceration, it was also proposed as a confounded factor. Herein, *H. pylori* was detected by the DNA amplification of the bacterial *phosphoglucosamine mutase* (*hpglm*M) gene in 299 homozygous Ala114 samples. The result showed that 74 ulcerative dyspeptic patients (cases) and 125 NUD patients (controls) were infected with *H. pylori* as the 294 bp *hpglm*M amplified products were presented in these samples (Figure 12). In this experiment, a clinical isolated *H. pylori* was used as a positive control. The amplification of the *GSTP* gene in co-purified human gastric DNA was also done in all samples (as an internal control). No DNA band presented when bacterial DNA was replaced by distilled water (a negative control).

6. Stratified analysis of an association between the GSTP Ile105Val genotypes and peptic ulcer

To clarify the effects of the confounded factors (sex, age, *H. pylori* infection) on the association between the GSTP genotype and the peptic ulceration, the stratified analysis was used for this purpose. Unfortunately, other factors such as cigarette smoking, alcohol, NSAIDs, food intakes were not included into this analysis because their data were not available. Table 21 demonstrates that both sex and age did not affect the association as their odds ratios did not reach the level of statistical significance. However, the *H. pylori* infection seemed to have an influence on the difference of the GSTP Ile105Val genotypes on the peptic ulceration. This was indicated by the statistically significantly increased odds ratio in homozygous Val105 genotype in the *H. pylori* infected patients.

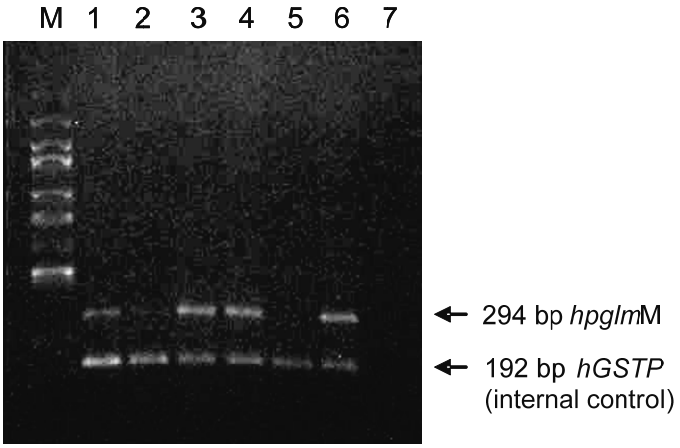

Figure 12 Detection of *H. pylori* in gastric biopsied specimens by DNA amplification of the *hpglm*M gene**:** Lane M = 100 bp DNA marker; lanes 1, 3, 4 and 6 = biopsies with *H. pylori* infection; lanes 2 and 5 = biopsies without *H. pylori* infection; lane 7 = negative control

Table 21 Analysis of an association between the GSTP genotype at codon 105 and a risk to peptic ulcer disease by stratified analysis

		GSTP genotype	Case (n =105)	Control (n =194)	Odds ratio (95%CI)	p-value
Sex	**Male**	Ile/ Ile	55	40	reference	reference
		Ile/ Val	16	22	1.049 (0.450 – 2.444)	0.099
		Val/ Val	6	2	2.182 (0.418 – 11.376)	0.467
	Female	Ile/ Ile	14	71	reference	reference
		Ile/ Val	12	58	0.529 (0.247 – 1.133)	0.911
		Val/ Val	2	1	10.143 (0.860 – 119.666)	0.084
Age	**< 40 yr.**	Ile/ Ile	19	55	reference	reference
		Ile/ Val	5	36	0.402 (0.138 – 1.173)	0.088
		Val/ Val	2	0	NA	NA
	≥ 40 yr.	Ile/ Ile	50	56	reference	reference
		Ile/ Val	23	44	0.585 (0.311 – 1.102)	0.096
		Val/ Val	6	3	2.240 (0.532 – 9.430)	0.314
H. pylori	**Positive**	Ile/ Ile	49	72	reference	reference
		Ile/ Val	18	51	0.519 (0.271 – 0.992)	0.046
		Val/ Val	7	2	5.143 (1.025 – 25.802)	0.039
	Negative	Ile/ Ile	20	39	reference	reference
		Ile/ Val	10	29	0.672 (0.274 – 1.651)	0.385
		Val/ Val	1	1	1.950 (0.116 – 32. 837)	1.000

NA = Not identified analysis