CHAPTER VI
DISCUSSION

There are a number of reports described the protein structure, enzymology and expression of enzymes in detoxification process, including glutathione S-transferases (GSTs) (Tsuchida and Sato, 1992; Rebbeck *et al*., 1997). GSTs are group of enzymes involving the metabolism and induction of known or suspected endogenous and exogenous compounds. Based on the observation that allelic variants of GSTs have different abilities to conjugate substances to glutathione, the roles of GST polymorphisms in cancers and some non-cancer diseases have been hypothesized. Average or increased GST activity may prevent some susceptible tissues from electrophilic toxic metabolizes by facilitating conjugation and their subsequent elimination. Decreased or deficient GST activity may cause poorer elimination of toxic compounds and, therefore, results in increased toxicity, leading to tumors or some other diseases (Ryberg *et al*., 1997; Harris *et al*., 1998).

Although most classes of GSTs were found to be polymorphic, particular interest was focused on the pi class GST (GSTP) because the gene coding for this isoenzyme is significantly over-expressed in several tumor tissues (Harries *et al*., 1997). Resently, there are many reports on the frequency of GSTP genotypes in different population and distribution of Ile105Val and Ala114Val genotypes was shown in Tables 6 and 7 in Chapter III. It is important to note that the frequency of Val114 gene was very rare in Asian population (including Thais in this study) but common in Caucasians. This indicated that the presence of the Val114 allele in the Caucasian population occurred before the divergence of the major racial groups and has been stable over this period.

Some reports demonstrated that GSTP was the main isoenzyme abundantly found in the stomach (de Bruin *et al*., 2000; Coles *et al*., 2002; Hoensch 2002). Therefore, the enzyme would play the major role to protect the gastric tissue from damage. In this study, the result showed an association between the GSTP genotypes and the ulcer dyspeptic disease. However, the stratified analysis revealed that, indeed, the main factor contributing to the difference of genotypes on the peptic ulceration was the *H. pylori* infection. Therefore, it is possible that the GSTP genotype did not

directly play a role as a main protective factor against the peptic ulceration. Moreover, it also supported that protection of the gastric mucosa from toxic substances involved other enzymes including glutathione peroxidase (GPx), γ-glutamyl transpeptidase (γGT) (Hirokawa and Kawasaki, 1995). The genetic polymorphism of these enzymes (and other peptides) might also play roles as either protective or risk factors to peptic ulcer.

Many studies examined the association between the GSTP genotypes and risk of several gastrointestinal diseases, particularly cancers (e.g. oral, esophageal, gastric and colorectal cancers), however, their results were contradictory. In gastric cancer, some studies found no association between GSTP genotype and the risk of the cancer (Mark *et al*., 2004; Katoh *et al*., 1999; Setiawan *et al*., 2001). Nevertheless, there was a report studied the relationship between chronic gastritis (precursor lesion for gastric cancer) and GSTP polymorphism (Setiawan *et al*., 2001). Similar to the result of the latter report, this project failed to find the association between GSTP genotype and the risk of peptic ulceration (that may develop to gastric cancer). This result indicated that the peptic ulcer could be influenced by some other factors, namely, external GST inducers, such as polycyclic aromatic hydrocarbons (PAHs) or foods that were suspected to contribute to different GST expression (Hoensch *et al*., 2002). Moreover, combination of other genes may involve the risk of the disease. Because GSTP, GSTM and GSTT are known to exhibit overlapping substrate specificity, it is possible that deficiencies of certain GST isoenzymes may be compensated by other enzyme isoforms (To-Figueras *et al*., 1999; Ryberg *et al*., 1997). However, Setiwan and co-workers (2001) observed no combined effects between GSTP and either GSTM or GSTT1 null genotype on the risk of either chronic gastristis or gastric cancer. This research group proposed that the carcinogen involving gastric carcinognesis are isoenzyme specific substrates that may not be shared among the GST isoenzymes (i.e., GST enzymes work independently), or there may be too small number of cases so that it limited an ability to estimate the GSTP effect precisely.

Studies in other gastrointestinal cancers that associated with the Val105 genotype include oral and colorectal cancers (Park *et al*., 1999; Katoh *et al*,. 1999; Ates *et al*., 2005). Park (2000) and Katoh (1999) suggested that GSTP Val105 may

play a role as a risk factor for oral cancer. Ates (2005) indicated that the risk of colorectal cancer increased when the putative high-risk genotype increased in combination with genotype of GSTM, GSTT null. However, Martinez and co-workers (2006) found a different result that GSTP Ile105 increased risk of colorectal and gastric cancers, whereas GSTP Val105 played a protective role for both cancers. Similar to the study of Martinez's group (2005), Jain and colleagues (2005) showed that GSTP Ile105 genotype in smokers and tobacco chewers appeared to associate with high risk for esophageal cancer. There are a few recent reports indicated that GSTP enzyme with Ile105 may be less active towards carcinogenic diol epoxide and benzo[alpha]pyrene compared to Val105 enzyme (Hu *et al*., 1997; Eaton and Bammler, 1999). Therefore, Ile105 allele of GSTP may play an important role in modulation of the cancer risk from tobacco usage. However, the difference was not statistically significant which suggested that GSTP Ile105Val polymorphism may have only marginal influence for conferring tobacco related risk for esophageal and oral cancers.

In addition, there are many diseases that were determined for their association with GSTP genotype as shown in Table 8. Some studies provided evidences that linked the GSTP Val105 genotype to increased risks for several cancers such as bladder, testicular, pharyngeal and oral cancers. Indeed, there were both association and no association between GSTP Val105 genotype and susceptibility to diseases (e.g. esophageal, lung, prostate and colorectal cancers). In lung cancer, for example, Ryberg (1997) and Miller (2003) showed that the enzyme with the Val105 allele has an elevated catalytic activity for PAH compared to that with the Ile105 allele. Many recent reports suggested that individuals with allele for low GSTP activity had high level of smoking related DNA-adducts in lung tissue and also had higher risk of lung cancer (Wang *et al*., 2003; Stucker *et al*., 2001; Miller *et al*., 2003). It is possible that a concomitant polymorphism in cytochrome P450 may play a dominant role in cancer susceptibility to PAH (Ryberg *et al*., 1997). Moreover, Hayes and Pulford (1998) noted that GST enzymes probably modulate the expression of other drug-metabolizing proteins. Thus, the polymorphism in GST may influence the other chemical defense mechanism, and indirectly confer the risk for develop cancer.

Interestingly, many non-cancer diseases were studied on their association with GSTP polymorphism (e.g. Parkinson's disease, Alzheimer's disease, glaucoma, cataract, asthma and schizophrenia). These diseases were known to relate to some factors that might be substrate for GSTP enzyme, for examples, oxidative stress, chemotherapeutic drugs or pesticides. Since, GSTP involved the detoxification of ROS (Sato *et al*., 1989), an alteration in GSTP activity would cause an increase in the level of oxidative stress, leading to the development of schizophrenia, glaucoma and cataract (Pae *et al*., 2003; Juronen *et al.*, 2000). Pae and co-workers (2003) also indicated that the negative finding for the association between GSTP genotype and schizophrenia should lead to some consideration. This group of investigators also reported that the GSTP polymorphism may not directly affect the development of schizophrenia or exert a very weak influence on the etiopathogenesis of the disorders. Thus, the presence of other unknown GSTP modulating factors and/or unidentified surrounding genes that may confer susceptibility to schizophrenia could also be taken into an account. However, they did not completely reject the association of GSTP with schizophrenia. The different effect of particular allelic forms of GSTs may result from their different abilities to resist the oxidative damage. Nishihara (1991) reported that oxidative stress conditions can selectively inactivate different GST isoenzymes and lead to a loss of ability to detoxify the harmful substances. The author proposed that individuals' genetic variants in GSTs might play important roles in their process.

Although the roles of GSTP genotypes on clinical outcomes are still controversial, the GSTP gene and genotype frequencies may be useful in a field of pharmacogenomics which is going to become more important in modern medicine in selecting appropriate drug and dosage for each patient. In this study, the gene frequencies of Val105 and Val114 in Thais are lower than those in the Caucasians, but similar to those in Asian populations (Tables 6, 7 and 10). This result is in consistence with many recent studies on drug-metabolizing enzymes which indicated that race-specific alleles can be the major factors in determining pharmacogenetic phenotype. For examples, Marahatta *et al* (2006) reported the resemblance of GST omega class (GSTO1*D140) gene frequency in Asian population. Oscason (2000) and Hein (2000) studied the gene frequency of human cytochrome P450 and arylamine N-acethyl transferase and found that the CYP1A1*4 allele was not observed in Africans while

the CYP2C19 allele (poor metabolizer) was common and specific in Asians and NAT2*14 allele (slow acetylator) appeared to be African-specific. Not only the gene of drug-metabolizing enzymes, the different gene frequencies in different racial groups were also found in genes of other enzymes, e.g., the blood coagulation factor XIIIA subunit (Okumura *et al*., 2003). This suggests that data of gene and genotype frequency from the Caucasians (or other races) could not immediately be applied to Thai of other Asian patients. Therefore, determination of the gene and genotype frequencies in population of different races is necessary.

In conclusion, the study on an association between the polymorphism of GSTP and the susceptibility to peptic ulcer disease was carried out. The result in this study showed an association between Ile105Val genotype and the peptic ulceration in Thai dyspeptic patients who were infected with *H. pylori.* This might be due to the fact that the association was influenced by the *H. pylori* infection. In addition, the sample size used in this study might be rather small. Therefore, to get more precise and accurate data in the future study, the sample size should be large. In addition, study on gene to gene and gene to environment interaction as well as other risk factors would be added into the study.

Analysis of data from a number of reports on association between GSTP genotypes and clinical pathology of many organ systems (table 8 in chapter III) indicated that the genotypes did not directly play either protective roles or risk factors in cancers and non-cancer diseases. This data confirmed that, besides the GSTP genotypes, there could be other factors such as genotypes of other enzymes, foods, drugs, and smoking etc. playing role in these diseases.