CHAPTER III
LITERATURE REVIEW

1. Peptic ulcer

Peptic ulcer disease is found all over the world. In most western countries, morbidity from duodenal ulcer is more common than from gastric ulcer, even though deaths from gastric ulcer exceed or equal those from duodenal ulcer. It has been estimated that 5-10% of individuals in the United States suffer from peptic ulcers during their life (Sonnenberg and Everhart, 1996). In contrast, the true prevalence and incidence of peptic ulcer among the Thai population have never been accurately assessed. During 1981 and 1988, the hospitalization rate for peptic ulcer cases throughout the country remained fairly constant at around 0.1% of total population (Wilairatana *et al.*, 1991). To date, although the cases decline, the diseases are still important because of death from gastric bleeding in some cases. Moreover, some studies indicated that chronic ulceration or prolonged inflammation may develop gastric cancer (Correa, 1996; Suzuki *et al*., 2006).

In general, the definition of peptic ulcer is a damage in mucosa lining the gastrointestinal tract such as the first part of duodenum, junction of antral and body mucosa in stomach and distal esophagus. In most cases, the ulcers are principally found in duodenum (duodenum ulcer) and stomach (gastric ulcer). The ulcers may be acute or chronic and may be complicated hemorrhage, perforation, obstruction due to fibrous strictures and malignancy. Acute peptic ulcers may develop from a part of acute gastritis, severe stress response or a result of extreme hyperacidity. Chronic peptic ulcers are most likely to occur where acid and pepsin first come into contract with a susceptible mucosa (Robbin *et al*., 1994). For many years, peptic ulceration has been thought to be a consequence of excessive acid production. However, this hypothesis is in a controversy. Individuals with gastric ulcers frequently have normal or even subnormal acid production, and over one-half of duodenal ulcer patients do not have hyperacidity. Conversely, many people who are hypersecretors of acid do not get ulcers. Furthermore, while most ulcers respond initially to anti-acid treatment, they frequently relapses. Discovery of *Helicobacter pylori* by Marshall and Warren (Marshall and Warren, 1984) elucidated that the bacterial infection was the main cause of the ulcers. This led investigators to study the potential role of the bacterial infection

in gastritis, peptic ulcer and gastric cancer. Then the concept of management of the ulcers was changed and the ulcers are now accepted as an infectious disease.

2. Risk factors of peptic ulceration

A number of etiological factors apparently implicated in the pathogenesis of peptic ulcers, but no single agent seems to be responsible. The main etiological factors that produce peptic ulcers are hyperacidity, *H. pylori* infection, NSAIDs, smoking and genetic factors. However, many recent studies showed that the use of NSAIDs and *H. pylori* infection are the main causes of peptic ulceration (Ramsoekh *et al*., 2005). In addition, infection with *H. pylori* was commonly found throughout the world and possibly linked to gastric cancer. Thereby, it has been classified as group 1 carcinogen by the World Health Organization (WHO) (Catherton *et al*., 1996; Taylor and Parsonnet, 1995; Cave *et al*., 1996). Blaser (1990) reported that *H. pylori* infection was detected nearly 100% in patients with duodenal ulcers, and frequently found in gastric ulcer patients. Furthermore, individuals who developed peptic ulcer were likely to have chronic *H. pylori* infection up to 10 years (Nomura *et al*., 1994).

3. *Helicobacter pylori*

3.1 Distribution of *Helicobacter pylori* in Thailand

Helicobacter pylori, a curved gram negative bacilli, is now recognized as a major cause of gastritis and peptic ulcer (Marshall and Warren, 1984; Graham, 1991) and is classified as carcinogenic factor for gastric cancer (International agency for research on cancer, 1994). *H. pylori* is well adapted to colonize in the stomachs of human and other vertebrates for the life span of their hosts. A large number of people around the world are reported to be infected with this organism and also have gastric inflammation. However, most infected individuals do not develop dyspepsia and other gastroduodenal disorders (Holcombe *et al*., 1992; Israel and Peek, 2001). Therefore, the diagnosis is usually overlooked at the early stage of infection.

The epidemiology of *H. pylori* infection has been studied for many years by both serological and histological methods. The incidence of *H. pylori* infection ranged from 40 to 75% (Dooley *et al*., 1989; Kachintorn *et al*., 1992; Arnantapunpong *et al*., 1999; Chinprasatsak *et al*., 1993; Ovartlarnporn *et al* ., 1998; Suwanagool *et al*., 1993; Atisook *et al*., 2003). Atisook *et al*., (2003) showed distribution of *H. pylori* infection in each region of Thailand by microscopic examination of gastric biopsied samples

from dyspeptic patients. The overall incidence of *H. pylori* in Thailand was 48.2%. The incidences of *H. pylori* infection in northeastern, northern and central parts were higher than those in eastern and southern regions (Figure 1). It is important to note that the lower-incidence regions (southern and eastern) were coastal areas.

3.2 Pathogenesis of *Helicobacter pylori* infection

Although gastritis is common among *H. pylori* infected individuals, only a fraction of those patients develop a clinically important outcome, such as peptic ulcer and gastric carcinoma (Dooley *et al*., 1989). It is wildly accepted that *H. pylori* is a main factor leading to peptic ulcer disease. The infection is found in 90% to 95% of patients with duodenal ulcer and 50% to 70% of the gastric ulcer patients. Recurrence of ulcer after successful eradication of *H. pylori* is about 2% to 4% per year, compared with up to 80% when the infection persists. Furthermore, the association of *H. pylori* with type B chronic atrophic gastritis, gastric adenocarcinoma and low-grade gastric MALT lymphoma has been reported (Howden and Hunt, 1998).

The mechanism of *H. pylori* infection that causes peptic ulcer can be described as a multistep process. Firstly, the organism have to pass through gastric acid barrier and enter the mucous layer (colonization). Then, they adapt and multiply under the environmental condition of the gastric mucus (persistence). Ultimately, *H. pylori* existing in the gastric mucosa can induce the inflammation and lead to various degrees of gastric tissue destruction. Figure 2 demonstrates the gastric consequence after *H. pylori* infection. After the bacteria attach to or translocate across the surface of the gastric epithelial cell, their cell wall components (lipopolysaccharides; LPS) stimulate macrophages which in turn release cytokines including IL-8, tumor necrosis factor (TNF), and IL-1. These cytokines stimulate gastric epithelium to express and release IL-8 which in turn stimulate neutrophils to release proteolytic enzymes and reactive oxygen species (ROS) leading to epithelial destruction and gastric ulceration (Crabtree *et al*. 1996).

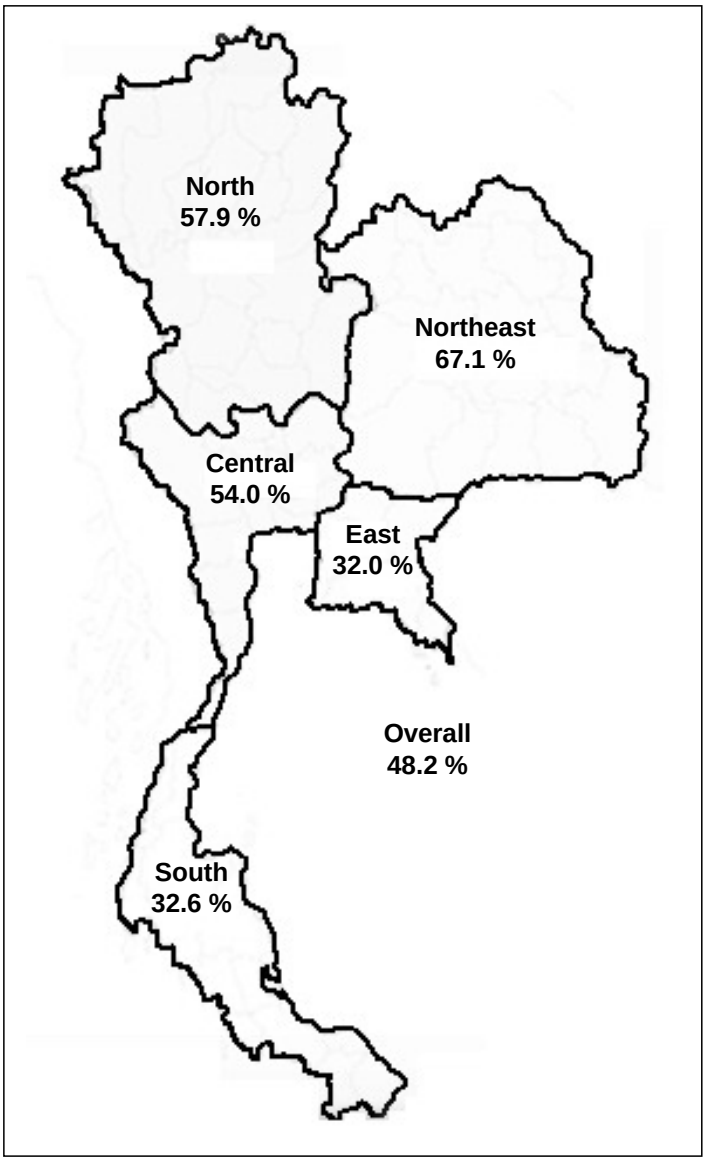

Figure 1 Distribution of *H. pylori* infection in each region of Thailand. (Modified from Atisook *et al*., 2003)

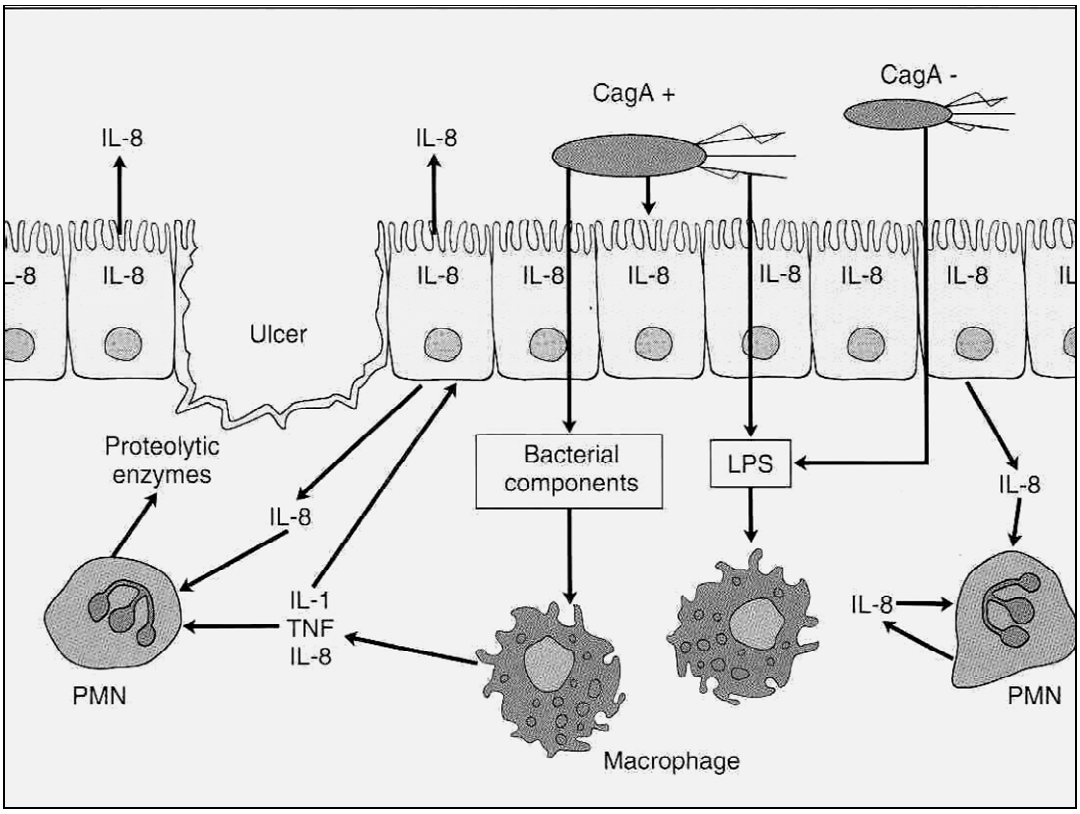

Figure 2 Secretion of cytokine after *H. pylori* attach the surface of the gastric epithelial cell (duplicated from Ferrero *et al*., 1997).

The production of a large amount of ROS, such as superoxide anion ($O_2^{\cdot -}$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot -}$) also cause gastric tissue damage, inflammation and cell proliferation. These reactive oxygen species (ROS) are detrimental to the integrity of tissues and mediate their injury. The mechanism of damage involves lipid peroxidation, which destroys cell membranes. This results in a release of intracellular components such as lysosomal enzymes, leading to further tissue damage. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and damage of DNA. The generation of superoxide anion and its mechanism of damage are well established in different models, but it has not been clear whether this radical is involved in gastric mucosal damage (Schraufstatter *et al*., 1988; Keshavarzian *et al*., 1992; Farnati *et al*., 1992). In addition, increased ROS levels were observed in *H. pylori* infected patients and it was believed that long term exposure to ROS contributes to a development of cancerous gastric cells. It was previously demonstrated that *H. pylori* induced gastric mucosal injury and inflammation might be caused by the oxidant-mediated expression of inflammatory cytokine interleukin-8 and inflammatory enzymes (Correa *et al*., 1995; Davies *et al*., 1994; Reider *et al*., 1997; Peek *et al*., 1997).

Although a large number of people around the world are reported to be infected with *H. pylori*, not all infected persons develop gastric pathology. This indicates the difference in host defence which possibly involves the individual's genetics.

4. Gastric mucosal defense

Ulceration is influenced by an equilibrium of damaging substances and host mucosal defense. The mucosal defense refers to the process that protects cell or tissue from various substances such as endogenous and exogenous toxins as well as bacterial products that can cause inflammation or ulceration. In general, human may not completely resist to damage induced by these substances, so the mucosal injury happens. However, a repair of this damage can quickly occur in the epithelium. The resistance of the mucosa would be enhanced when toxic substances are present in the

lumen of stomach. The activity of mucosal defense is organized in various processes. The first level of defense consists of the secreted factors in the lumen, including acid, mucus, bicarbonate and antibacterial substances (e.g. immunoglobulin). The second level of defense is the epithelium which resists to acid-induced injury and acts as a barrier to passive diffusion. Damaged epithelium would be repaired quickly through the processes that involve migration of healthy cells over the injury region. The mucosal microcirculation is the third level of defense that is operated by the nervous system. Diffusion of acid or toxins into the mucosa results in a sensory nerve-mediated elevation of mucosal blood flow that is necessary for limiting the damages and facilitating repairs. The blood dilutes and/or neutralizes the acid/toxin and prevents it from accumulation in the mucosal tissue. The fourth level of defense is the mucosal immune system. This system consists of various cells, such as mast cell and macrophage which respond to the entry of foreign materials into the mucosa and alter an inflammatory response. This level of the defense may produce reactive oxygen species from chronic inflammation. The final level of mucosal defense starts when mucosal damage extends deeper than the superficial epithelium. In this circumstance, the mucosa is able to undergo repair that includes growth and re-development of gastric glands, renewed innervation by the extrinsic and intrinsic nervous systems and re-establishment of the microcirculation through angiogenesis. Nevertheless, the ulcer can develop when the mucosal defense are imbalance to the damaging substances (Wallace *et al*., 1997).

There are many studies on mucosal defense mechanism against reactive oxygen species in gastrointestinal tract. The reduced form of the tripeptide thiol, glutathione (GSH) is one of the major endogenous defense mechanisms against oxidative stress. GSH acts as a scavenger of free radical and toxic substances that produce in the gastrointestinal tract (Shenoy *et al*., 1992; Grisham *et al*., 1992; Pihan *et al*., 1987). GSH protects gastric mucosa cells from damage by nitric oxide and exhibits other beneficial effects as co-substrate for glutathione S-transferase (GSTs) and glutathione peroxidase. Glutathione peroxidase catalyzes the degradation of organic or lipid peroxide, whereas GSTs catalyze conjugation of GSH to a variety of carcinogens and toxins to form less toxic compounds (Reinemer *et al*., 1991). The GST and GSH concentration are studied in many gastrointestinal diseases and cancers.

Diminished levels of GSTs and GSH have been observed in several gastrointestinal cancers and precancerous conditions, including Barrette's metaphasia of esophagus (Zhang *et al*., 1992) and colon carcinoma (Sinning *et al*., 1993). The GST activity and GSH concentration in gastrointestinal tract are wildly studied as summarized in Table 1.

Table 1 also shows that GSH concentration and GST expression were decreased in *H. pylori* infected mucosa comparing to non-infected tissues (Verhulst *et al*., 2000; Shirin *et al*., 2001; Hoensch *et al*., 2002; Baek *et al*., 2004). These results indicated that the loss of GST may increase a risk of developing gastric lesion in *H. pylori* infected patients (Wang *et al*., 2000). Moreover, an expression of each class of GSTs such as GSTP, GSTA and GSTM was identified in gastrointestinal tract. It also demonstrated that the expression level of GSTP decreased from stomach to colon. Conversely, GSTA1 and GSTA2, two subclasses of alpha class, expressed at low level in stomach and colon but high level in duodenum and small intestine. In addition, GSTM1 and GSTM3 appeared to express at low level in gastrointestinal tract. These results indicated that GSTP is the major GST in stomach whereas the major GSTs in duodenum and small intestine are GSTA1 and GSTA2 (Coles *et al*., 2002; Hoensch *et al*., 2002). Furthermore, a few studies indicated that the risk of gastric cancer was increased when low or no expression of GSTP was combined with *H. pylori* infection (Wang *et al*., 2002).

5. Glutathione S-transferases (GSTs)

5.1 Role of GSTs in enzymatic detoxification

Living organisms defend themselves from toxicants which present in the environment or endogenous xenobiotics through detoxification system. In general, cells have developed an enzymatic antioxidant pathway against ROS generated during the change of superoxide anion ($O_2^{\cdot -}$) to hydrogen peroxide (H_2O_2) by superoxide dismutase (SOD) and the conversion of H_2O_2 to H_2O by glutathione peroxidase (GPx) or catalase (Warner *et al*., 1994; Michels *et al*., 1994; Fridorvich *et al*., 1997). The imbalance between these two reactions is critical as it can cause an accumulation of $O_2^{\cdot -}$ or H_2O_2. In the presence of Fe^{++}, H_2O_2 can be converted to $OH^{\cdot -}$ (Fenton reaction) as shown in Figures 3 and 4.

Table 1 Recent studies on GST activity and GSH concentration in gastrointestinal tract

Summary of study	Reference
GSTP expressed at higher level than GSTA and GSTM, respectively, in tumor tissue.	Peter *et al*., 1990
GSH concentration and GST activity were lower in *H. pylori* infected patients than those who are *H. pylori* negative.	Verhulst *et al*., 2000
GSTA, GSTT and GSTP highly expressed in all gastrointestinal tissues.	de Bruin *et al*., 2000
Decreased GSH concentration was observed in *H. pylori* infected patients.	Shirin *et al*., 2001
GSTP expressible rate decreased from normal gastric mucosa to intestinal metaphasia (IM) to cancer.	Wang *et al*., 2002
Total GST activity and GSTA content were low in antrum but high in duodenum, whereas GSTP content was high in antrum but low in duodenum.	Hoensch *et al*., 2002
GSTP expressed at higher level in stomach than colon. GSTA1 and GSTA2 expressed in both duodenum and small intestine. GSTM1 and GSTM3 expressed at low level in gastrointestinal tract.	Coles *et al*., 2002
The proteomic study in *H. pylori* infected human gastric mucosa indicated decreased GST.	Baek *et al*., 2004

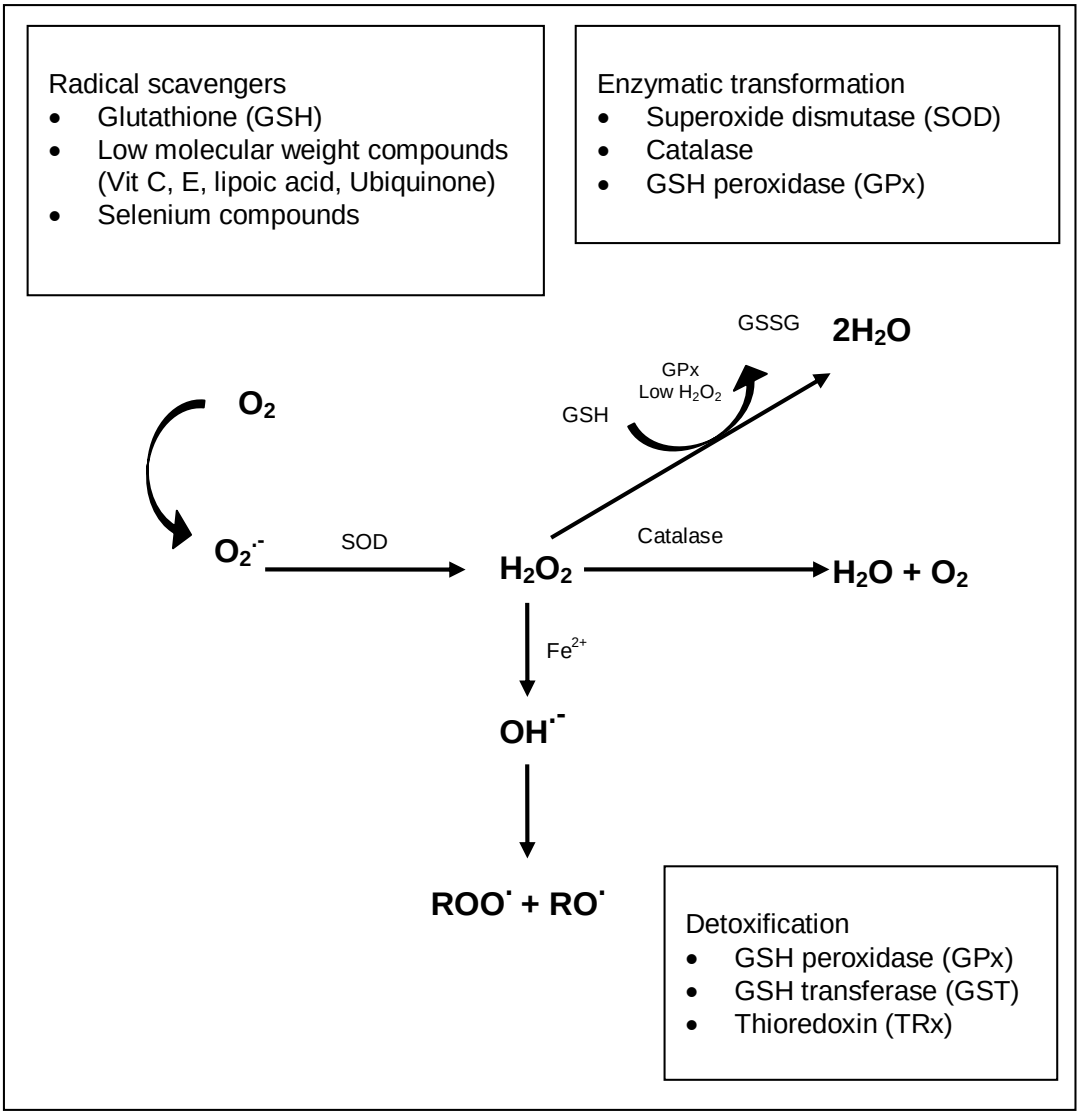

Figure 3 The cellular antioxidant defenses in cellular. $O_2^{\cdot -}$, superoxide anion; H_2O_2, hydrogen peroxide; $OH^{\cdot}$, hydroxyl radical (modified from Bandyoradhyay *et al*., 1999; http:// biologie.univ-mrs.fr/upload/p76/Stress__tissus_2006.pdf)

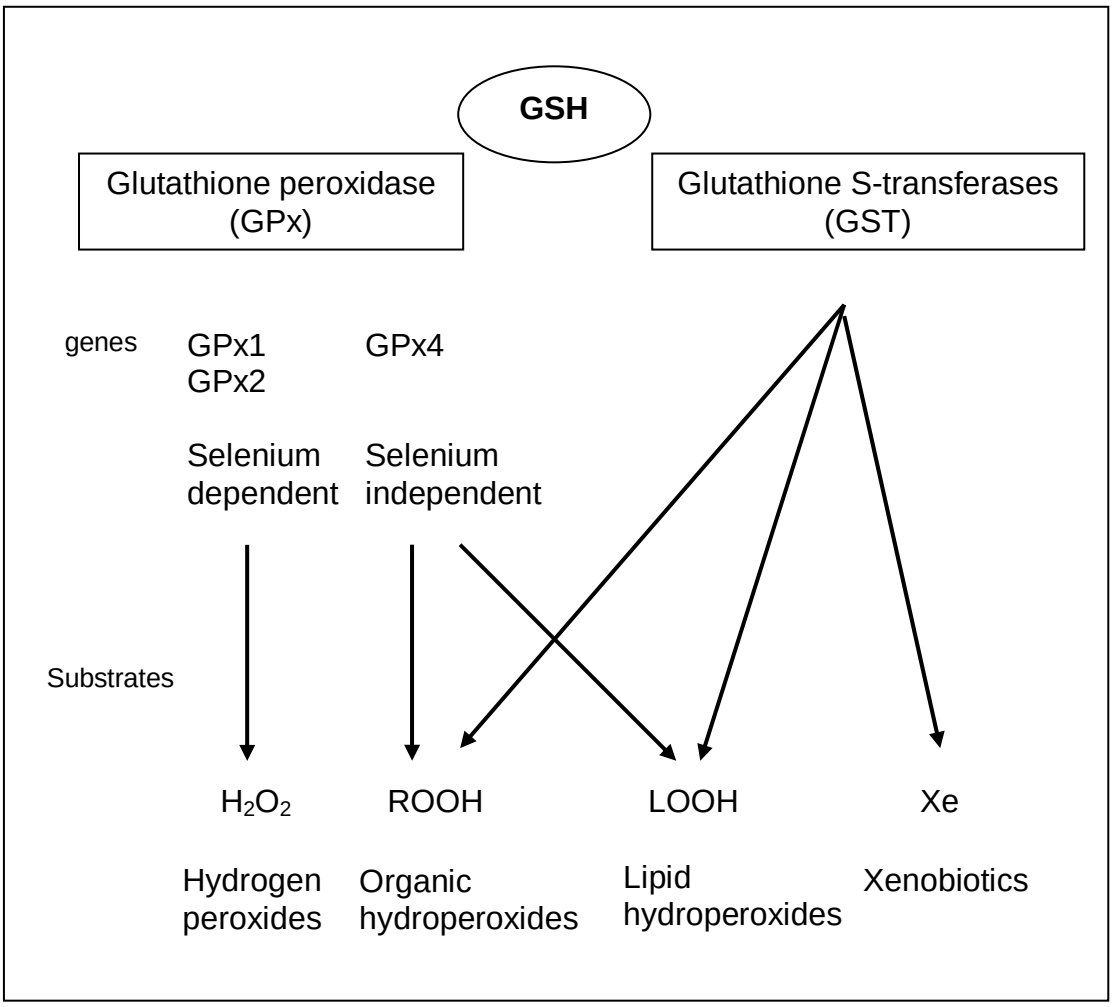

Figure 4 Detoxifying functions of GSH-dependent enzymes (modified from Bandyoradhyay *et al*., 1999 and http://biologie.univ-mrs.fr/upload/p76/Stress__tissus_2006.pdf)

In addition, some reducing agents (reduced glutathione; GSH, vitamin C and β-carotene) act as non-enzymatic scavengers of ROS. Collectively, these substances provide a first line of defense against free radicals. The second line is provided by some enzymes such as aldo-keto reductase, aldehyde dehydrogenase and glutathione S-transferase, which have been found to be inducible by several pro-oxidant agents (Hayes and McLellan, 1999).

The enzymatic detoxification of xenobiotics was classified into three distinct phases as shown in Figure 5. Phase I and II involve the conversion of lipophilic and non-polar xenobiotics into more water-soluble and less toxic metabolizes, which can be excluded more easily from the cell (phase III). Phase I is catalyzed mainly by the cytochrome P450 system that is responsible for a range of reactions, such as oxidation, reduction and hydrolysis of substrate. Phase II enzymes conjugate xenobiotics with various endogenous substrates, such as glucuronide, glutathione and sulfate to produce hydrophilic products which are excreted easily from the cell. The GSH conjugation which is catalyzed by the GSTs, is the major phase II reaction in many species. The GSH-xenobiotic conjugated products are more hydrophilic to diffuse freely from the cell, and have to be pumped out actively by transmembrane ATPase such as the GS-X pump, which is phase III. (Sheehan *et al*., 2001; Salinas and Wong, 1999)

Glutathione S-transferases (GSTs; EC.2.5.1.18) are superfamily of enzymes that play an important role in phase II in cellular defense against various electrophilic compounds. They catalyze the nucleophilic addition of glutathione (γ-glutamyl-cysteinyl-glycine) to electrophilic centers in organic compounds. The resulting conjugated products are more water-soluble in order to facilitate their elimination. This reaction is one of the early steps along the mercapturic acid pathway (Figure 6) in which hydrophobic xenobiotics are inactivated and eliminated from the organism (Habig *et al*., 1974). The catalytic strategy can be divided into several steps involving binding of substrate to enzyme, activation of GSH by thiol deprotonation, nucleophilic attack by thiolate at electrophilic center, product formation and product release (Armstrong *et al*., 2001; Caccuri *et al*., 1996; 1997; 1998). Through these reactions, GSTs play an important role in the protection of cells from several environmental carcinogens and products of oxidative stress that can cause cytotoxic or genotoxic damage (Hayes and Pulford, 1995; Hayes and Strang, 1995; Pickette *et al*., 1989; Cole *et al*., 1990).

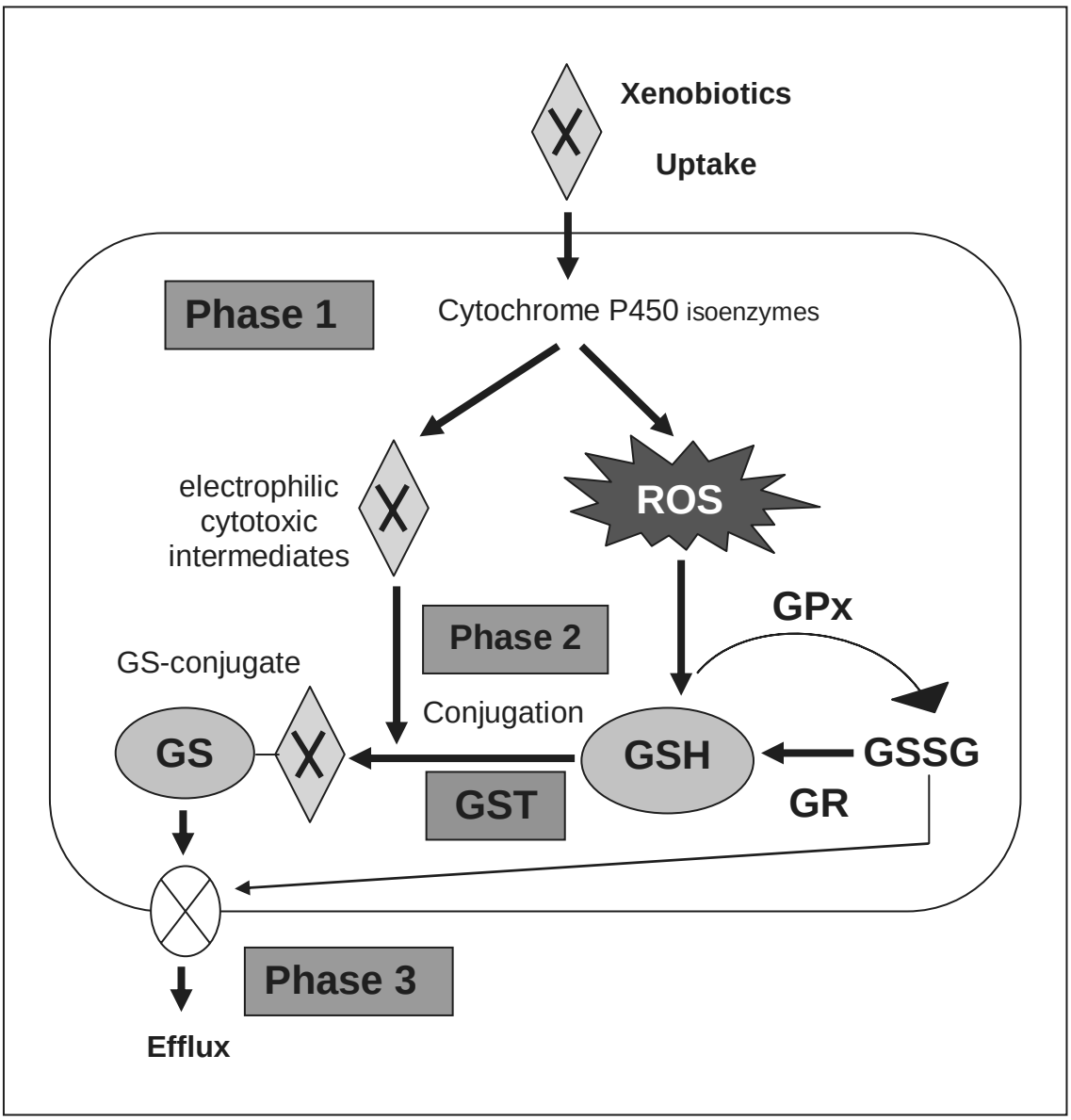

Figure 5 Xenobiotic detoxification by glutathione S-transferase (Duplicated from Sheehan *et al*., 2001)

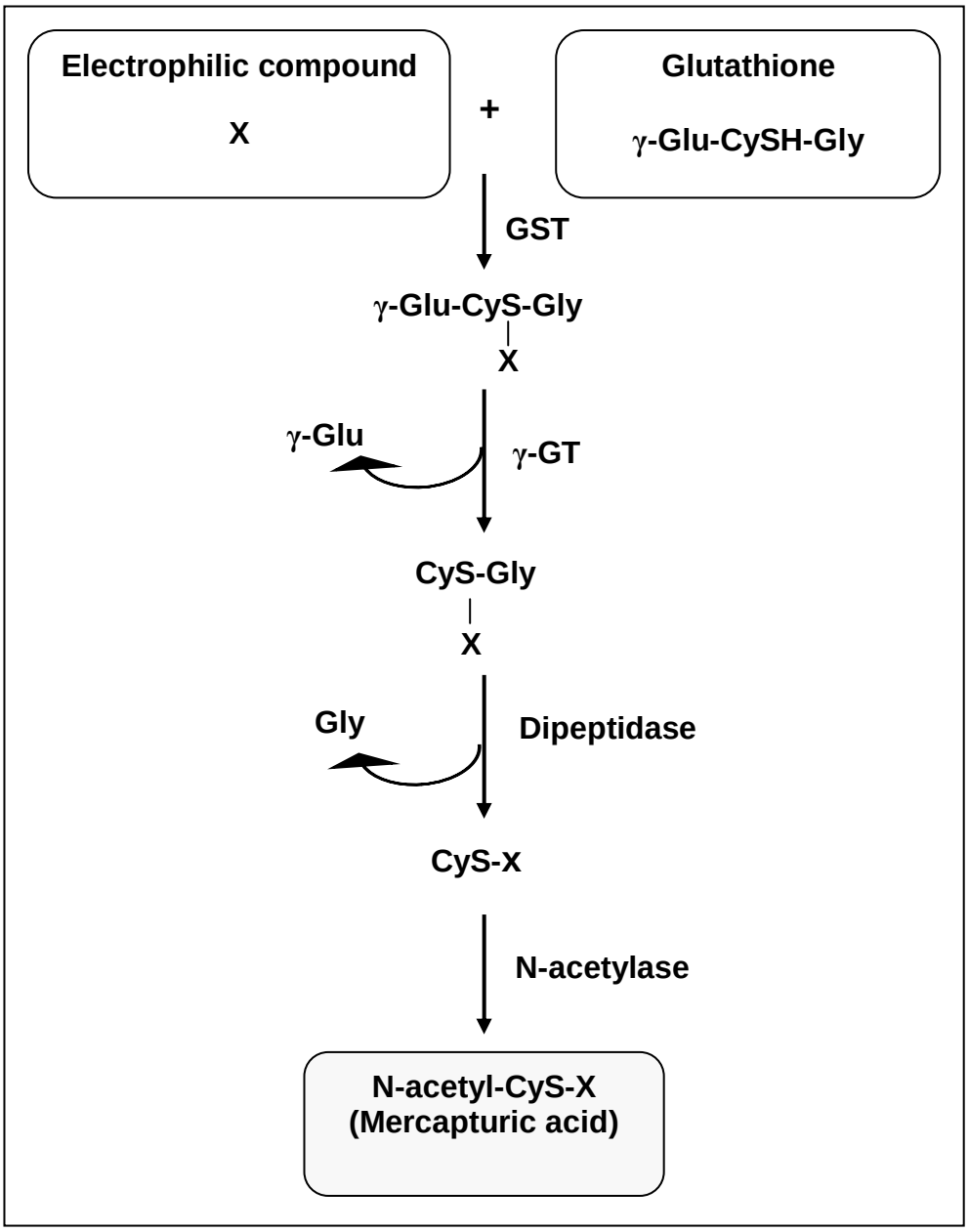

Figure 6 Mercapturic acid pathway (Duplicated from http://biologie.univ-mrs.fr/upload/p76/Stress__tissus_2006.pdf)

5.2 Classification of GSTs

GSTs are distributed in most aerobic eukaryotes and prokaryotes such as human, animals, plants, insects, parasites, yeasts, fungi and bacteria. They present in cytoplasm, membrane of mitochondria, microsome and nucleus. To date, the GST enzyme family has been divided into a number of classes based on many criteria, including amino acid/nucleotide sequences, immunological/kinetic properties, tertiary/quaternary structures and structural difference around the active site and at the inter-subunit interaction in crystal structure. The separation of GST classes by various criteria was summarized in Table 2. Although there are no clear criteria, the sequence similarity is generally accepted that GSTs share greater than 70% identity within the same class and those with less than 30% identity are assigned to separated class. Some reports demonstrated the use of immunoblotting techniques in identifying the tissue-specific expression of each GST class. Moreover, some kinetic properties such as substrate specificities and inhibitor sensitivities can also sometimes be used to distinguish different GST classes. The ability to form dimer of the enzyme was also used to identify the tertiary and quaternary structures of each GST class.

Human GSTs can be classified into six classes; namely, alpha, mu, pi, theta, sigma and omega, based on substrate specificity and primary structure (Mannervik *et al*., 1985; Hayes and Pulford, 1995; Armstrong, 1997; Hayes and McLellan, 1999; Sheehan *et al*., 2001; Board *et al*., 2000). Figure 7 shows the phylogenetic tree illustrating the diversity of GSTs and the relationship between classes. Branch lengths correspond to the estimated evolutionary distance between protein sequences. Therefore, GSTM (mu), GSTS (sigma), GSTP (pi) and GSTA (alpha) would be in the same group. Figure 7 also shows that the distance between GSTP and GSTA were closer than GSTM and GSTS classes. Figure 8 illustrates the ribbon representation of GST subunit structure in each class. The GSTs specifically to mammals (alpha, mu, pi and sigma) are in the same group. The plant specific enzymes are phi and tau, the structure of the latter class has yet to be reported. Bacterial specific enzyme is beta. The theta and zeta GSTs have counterparts in both animals and plants. Although there is little sequence similarity between enzymes of different classes, there is significant conservation in overall structure.

Table 2 Classification of glutathione S-transferase (Modified from Sheehan *et al*., 2001; Chauhan *et al*., 2003)

Classification criteria		Class	Reference
Primary structure	More than 70% protein sequence homology	Alpha/Mu/Pi	Mannervik *et al*., 1985
		Theta	Pemble *et al*., 1996
		Kappa	Pemble *et al*., 1996
		Omega	Board *et al*., 2000
Immuno-blotting	Recognition by antibodies against solvent accessible features	Alpha/Mu	Hayes and Mentle, 1986
		Rat GST	Blocki *et al*., 1992
		Insect classes I and II	Fournier *et al*., 1992
		Fasciola hepatica GST	Creaney *et al*., 1995
Kinetic property	Substrate specificity/affinity	Alpha/Mu/Pi	Mannervik *et al*., 1985
		Mu	Rowe *et al*., 1998
		Theta	Meyer *et al*., 1991
	Inhibitor sensivity	Alpha/Mu/Pi	Mannervik *et al*., 1985; 1988
Tertiary structure	Active site (catalytically essential residues at N-terminus)	Alpha/Mu/Pi	Dirr *et al*., 1994
		Theta	Ji *et al*., 1992
		Omega	Borad *et al*., 2000
		Kappa	Pemble *et al*., 1996
		Sigma	Ji *et al*., 1992
		Beta	Rossjohn *et al*., 1998
Quaternary structure	Ability to hybridize into dimeric form	Mu/Alpha	Hayes *et al*., 1981
		Alpha/Mu/Pi/Theta	Dirr *et al*., 1994; Wilce *et al*., 1994
		Beta	Rossjohn *et al*., 1998

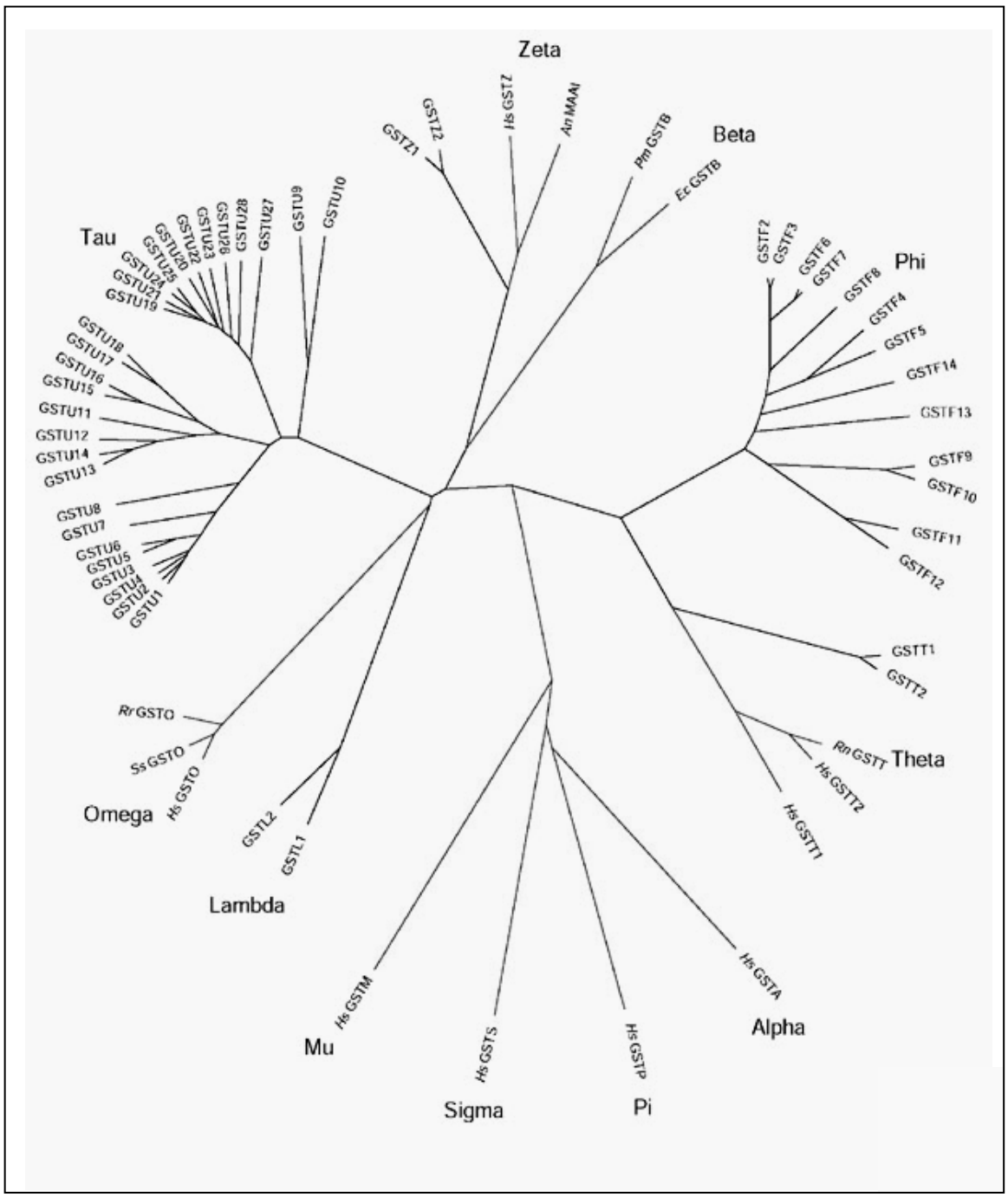

Figure 7 Phylogenetic tree of GSTs (*Hs*, *Homo sapiens*; *Rr*, *Rattus norvergicus*; *Ss*, *Sus scrofa*; *An*, *Aspergillus nidulans*; *Pm*, *Proteus mirabilis*; *Ec*, *Escherichia coli*) (Dixon *et al*., 2002)

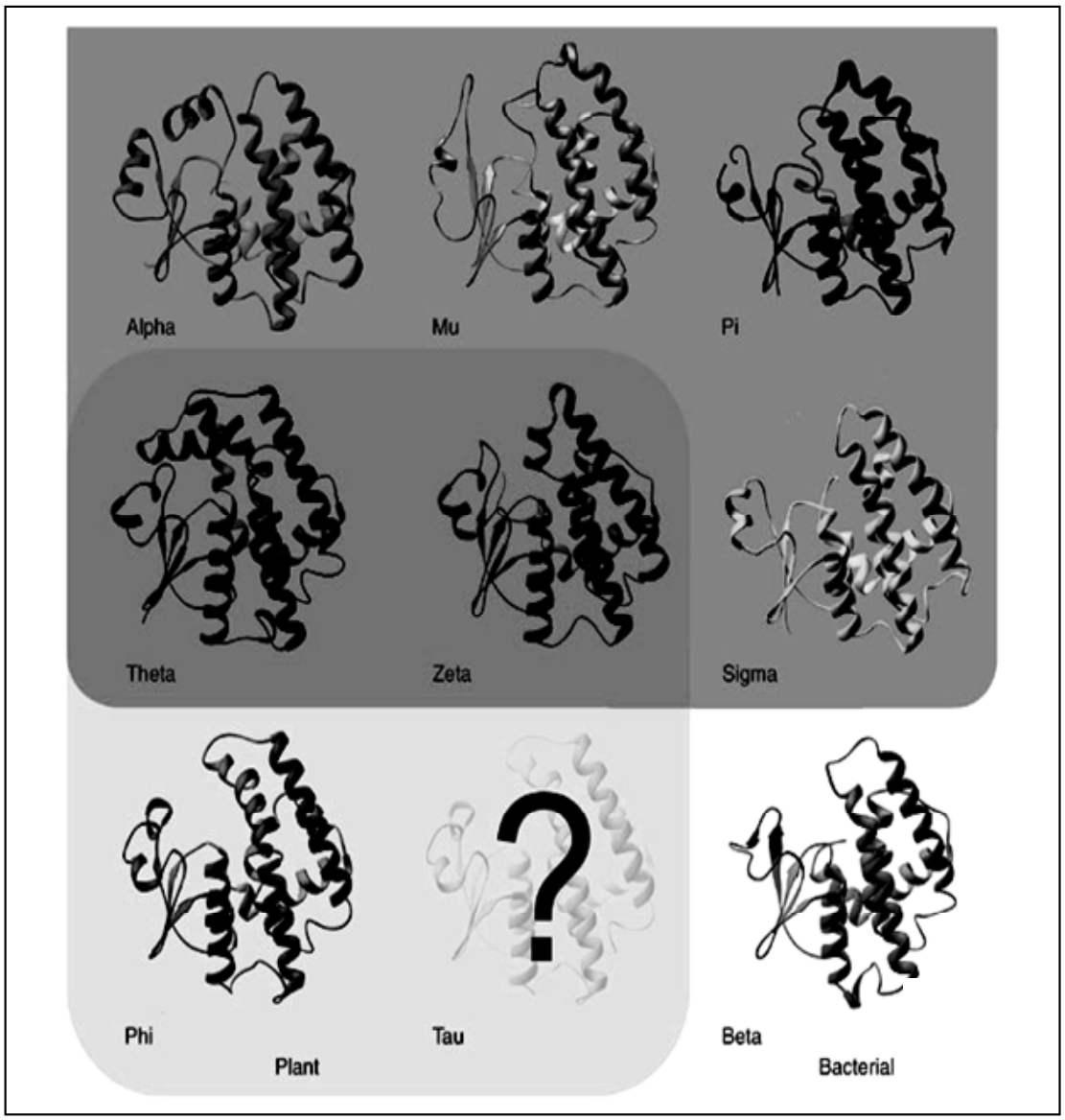

Figure 8 Ribbon structure of GSTs (Dixon *et al*., 2002)

The mass of the dimeric GSTs is ~50 kDa and subunits within the same class can combine to form either homo- or heterodimer (Mannervik *et al*., 1982; Stockman *et al*., 1985). Each subunit contains an active site and two domains: N-terminal domain (domain I) and C-terminal domain (domain II). There are at least two ligand-binding sites per subunit: the glutathione-binding site (G-site), which is very specific for GSH, and hydrophobic substrate-binding site (H-site), which can bind a large variety of different electrophiles (Mannervik *et al*., 1985). The G-site is constructed mainly from residues of the N-terminal domain whereas the H-site has major contribution from the C-terminal domain. While the properties of the amino acid residues making up the G-site are generally conserved among different classes, the residues forming the hydrophobic substrate-binding pocket become greatly diverse. Since the structure of the H-site determines the substrate specificity of the particular GSTs, diversity in the H-site gives the GST family the ability to catalyze reactions on a large number of structurally different substrates.

Although these GST classes have very similar overall polypeptide folds (see Figure 8), each class is little different in part of the active site and at the C-terminus. For alpha and theta classes, the C-terminus forms by α-helical structure that is active site and plays an important role in catalysis through the control of product release. The C-terminus form of mu class is μ-loop that covers the active site. Another active site of mu and pi classes is found in the segment that links strand β2 to helix 2 of the proteins (Wilce *et al*., 1994).

5.3 Substrate-specificity of GSTs

A variety of chemicals serve as substrates for GSTs. In each class, substrate specificity may be the same or different. For example, chlorinated nitrobenzenes (e.g. 1-chloro-2-4-dinitrobenzene; CDNB and 1,2-dichloro-4-nitrobenzene; DCNB) have served as standard substrates for nearly all GSTs. While, theta class does not catalyze this reaction. The specific activities toward CDNB and DCNB of most GSTs vary greatly between different isoforms. GSTT1 catalyzes the activation of small bi-functional electrophiles such as dichloromethane, ethylene dibromide and butadine diepoxide (Their *et al*., 1996). GSTA4 has high activity towards endogenous products of lipid peroxidation such as 4-hydroxy-2-nonenal and may play an important

physiological role in protecting against oxidative stress induced by endogenous lipid peroxides (Hubatsch *et al*., 1998). The trans-isomer of stibene oxide (TSO) is conjugated by GSTM1 and therefore this epoxide substrate serves as a selective marker for this mu class (Seidegard *et al*., 1989). Moreover, the carcinogen epoxide of benzo(a) pyrene (BPDE) is efficiently detoxified by GSTs, with high and selective activity of GSTP (Hu *et al*., 1997; Sundberg *et al*., 1998). The diuretic ethacrynic acid (ECA) is a good substrate and also a good inhibitor for some GSTs (Awasthi *et al*., 1993; Ploemen *et al*., 1993). This substrate has been use as a selective marker for GSTP class, although other GSTs also exhibit relatively high ECA activities (Table 3). Furthermore, GSTP is an interesting enzyme as its binds to reduced glutathione and involves the detoxification of ROS (Sato *et al*., 1989). A recent report suggested that GSTP prevents H_2O_2-induced DNA damage by scavenging the lipid-peroxide modified DNA (Kamada *et al*., 2004). GSTO class is not active against the standard GST substrate, CDNB, but it catalyses monomethylarsonic acid and dehydroascorbic acid (Yin *et al*., 2001; Schmuck *et al*., 2005).

The GST expression is different among tissues (Table 3) and not all GST classes express in every tissue. This possibly results from inter-individual genetic differences, diet and xenobiotic modulation, so it is difficult to accurately predict the extent of expression of any *GST* gene in a given tissue. However, immunoblotting and HPLC-electrospray mass spectrometry subunit analysis of GST class have provided some information on relative expression of different class of GST in human tissue (Hayes and Pulford, 1995; Listrowsky *et al*., 1998; Rowe *et al*., 1997). These investigators found that GSTA highly expressed in liver, kidney and testis. GSTT predominantly expressed in liver and kidney, to lesser extent to other organs and only at relatively low level in lung. In contrast, GSTM variously expressed in different tissue. For example, GSTM2 expressed at the highest level in brain and hardly found in liver, whereas GSTM1 expressed at the highest level in liver. Interestingly, GSTM3 expressed almost uniquely in this tissue (Listrowsky *et al*., 1998). GSTP expressed in relatively high level in brain and lung but not liver (Sheratt *et al*., 1997). Furthermore, the regulation of GSTP is of interest because its expression is significantly increased in many human tumors, human cell lines (Sato *et al*., 1989). On the other hand, expression of omega class (GSTO1 and GSTO2) is abundant in wide range of normal tissues except heart, which has low level of GSTO2 expression (Yin *et al*., 2001; Whitbread *et al*., 2003).

Table 3 Typical substrate and tissue specific expression of GSTs (Eaton and Bammler, 1999; Whitbread *et al*., 2003; Yin *et al*., 2001; Schmuck *et al*., 2005)

Class		Preferred substrate*	Primary tissue
Alpha	GSTA1	CDNB (moderate) DCNB (low) CHP (moderate-high) ECA (low-moderate)	liver, testis > kidney, adrenal > pancrease > lung, brain > heart
	GSTA2	CHP (high), CDNB, DCNB (moderate) ECA (low-moderate)	liver, pancrease, testis > kidney > adrenal >brain, lung, heart
	GSTA3	Unknown	pacenta (partial cDNA)
	GSTA4	4-HNE (high) ECA (moderate) CDNB, CHP (low)	small intestine > spleen
Mu	GSTM1	TSO (selective) CDNB (high) DCNB, ECA (moderate) CHP (low)	liver > testis > brain, adrenal, kidney, pancreas > lung, heart
	GSTM2	Catecholamine, quinines CDNB, DCNB, ECA (high) CHP(low)	brain > testis > heart > pancreas > kidney > adrenal > lung, liver
	GSTM3	CDNB, DCNB, ECA, CHP (low)	testis > brain, spleen > others
	GSTM4	CDNB, DCNB, ECA, CHP (low)	liver, skeletal muscle > heart, brain > pancreas > lung, kidney, placenta
	GSTM5	CDNB (moderate)	brain, testis, lung
Pi	GSTP1	BPDE, ECA (high) CDNB (moderate) DCNB, CHP(low)	brain > lung, heart, testis > adrenal, kidney, pancreas >liver
Theta	GSTT1	CHP(moderate-high) ECA(low) Dichromethane, EPNP, CDNB	kidney, liver > small intestine > brain, spleen, prostate, pancreas, testis > heart, lung
	GSTT2	CHP(high) 1-naphthyl sulfate, CDNB	liver
Omega	GSTO1	Monomethylarsonic acid Dehydroascorbic acid	liver, colon, heart, ovary, pancreas, prostate, spleen
	GSTO2	Dehydroascorbic acid	liver, kidney, skeletal, muscle, heart

* CDNB (1,2,4-dinitrobenzene) activities: high >150, moderate 50-150, low < 50 μmol/min/mg.
DCNB (1,2-dichloronitrobenzene) activities: high > 2, moderate 0.5-2, low < 0.5 μmol/min/mg.
ECA (ethacrynic acid) activities: high > 1, moderate 0.1-1, low < 0.1 μmol/min/mg.
CHP (cumene hydroperoxide) activites: high > 2, moderate 0.5-2, low < 0.5 μmol/min/mg.
EPNP (1,2-epoxy-3-(ρ-nitrophenoxy) propane)

5.4 GST polymorphism

The balance between metabolic activation and detoxifying pathway differs among individuals and is thought to affect cancer susceptibility. This emphasizes genetic variations in metabolic enzymes, including GSTs. Numerous polymorphisms were discovered in the genes encoding GSTs. Among them, GSTM, GSTT and GSTP genotypes have been studied during recent years for their potential roles in determining individual susceptibility to environmental factors induced diseases, particularly cancers (Hirvonen *et al*., 1999). Homozygous deletion of the *GSTM* or *GSTT* gene causes a lack of enzyme activity. The reduction of the activity is also caused by the polymorphism in the *GSTP* gene. The GSTM or GSTT null genotype and the variant GSTP allele may increase disease susceptibility due to decreased detoxifying potential (Hayes and Strange, 2000; Ali-Osman *et al*., 1997; Watson *et al*., 1998; Harries *et al*., 1997; Ryberg *et al*., 1997).

6. Glutathione S-transferase class pi (GSTP)

To date, many classes of GSTs have been reported and their more details can be cited elsewhere (Mannervik *et al*., 1985; Hayes and Pulford, 1995; Armstrong, 1997; Hayes and McLellan, 1999; Sheehan *et al*., 2001; Board *et al*., 2000). Herein, detail of the pi class GST was briefly reviewed. In comparison to other classes, the pi class has been discovered quite recently (Lo *et al*., 1997; Hayes and Pulford, 1995; Ali-Osman *et al*., 1997). Pi class is one of the most interesting GSTs in terms of evolution, biochemistry, polymorphism in human, ethnical differences and influence on human cancer risk. The pi class is so far one of the best characterized GSTs regarding to its role in the process that occurs between an exposure to mutagen and an induction of DNA damage. Furthermore, it has been recently reported that GSTP prevents H_2O_2-induced DNA damage by scavenging the lipid-peroxide modified DNA (Kamada *et al*., 2004).

6.1 Chromosome localization, gene structure and allelic variant of GSTP

The *GSTP* gene is a single gene located on chromosome 11. The *GSTP* gene spans ~3 kb and encodes 210 amino acids in seven exons. The promoter region contains a TATA box, two SP1 site, an insulin response element and antioxidant

response element in an AP1 site (Lo *et al*., 1997) (Figure 9). The *GSTP* gene can be induced by oxidative stress and this response may be mediated via NF-kappa B response element (Hayes and Pulford, 1995). Functional AP-1 and SP-1 response elements have also been identified in the 5' regulatory region of the *GSTP* gene. Post-transcriptional mechanism such as mRNA stability is also involved in regulating GSTP protein level (Moffat *et al*., 1994). Regulation of expression of the *GSTP* gene may also be influenced by the methylation status a CpG island in the regulatory region of the gene (Jhaveri and Morrow, 1998).

Human GSTP variants have been identified by heteroduplex analysis of genomic DNA and DNA sequencing as summarized in Table 4. However, most studies carried out in exons 5 and 6 of the gene. A functional polymorphism at codon 105 in exon 5 of the *GSTP* gene has been found. This polymorphic allele differs by a single base pair substitution (adenine to guanine) at nucleotide 313 resulting in an amino acid substitution (isoleucine to valine). Furthermore, study on biophysical characteristics of this polymorphism revealed that the valine variant has lower enzymatic activity and detoxification capability. The polymorphism in the exon 6 was found to be the transition (cytosine to thymine) at nucleotide 341, resulting in an amino acid change (alanine to valine) in codon 114 (Harries *et al*., 1997; Hu *et al*., 1998). Regarding to these two polymorphisms at the GSTP locus, the enzyme may be grouped into four allelic variants, GSTP1*A to D, that differ structurally and functionally and GSTP1*A acts as the common allele. Two sites in DNA sequence are variable and characterized by A to G transition at nucleotide 313 (found in both GSTP1*B and GSTP1*C) and C to T at nucleotide 341 (found in GSTP1*C and GSTP1*D). These groups of GSTP variants were summarized in Table 5 (Ali-Osman *et al*., 1997; Heenan *et a*l., 1997).

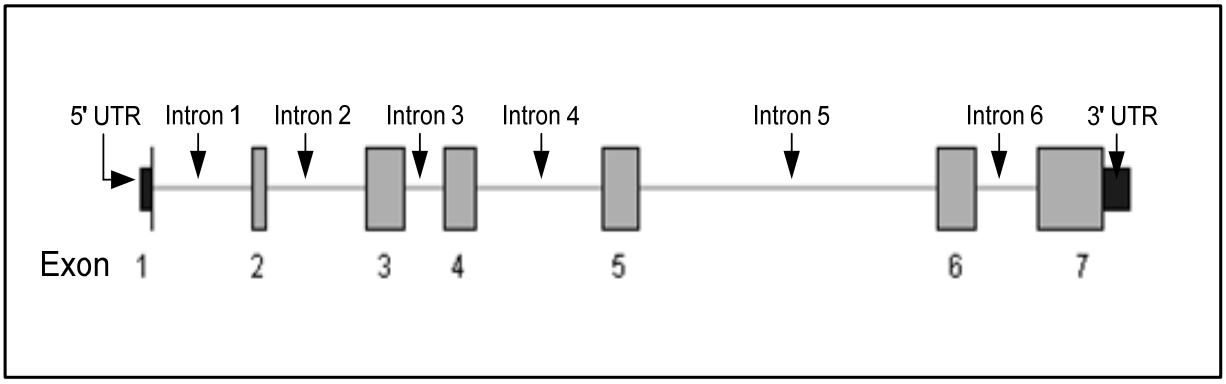

Figure 9 *GSTP* gene arrangement (http://atlasgeneticsoncology.org/Gene/GC_GSTP1.html).

Table 4 DNA polymorphism in the *GSTP* gene (Lin *et al*., 2003)

Gene region*	**Nucleotide change**	**Amino acid change**	**Reference**
Intron 4 (nt.882)	C to A	-	NCBI SNP ID rs.762803
Exon 5 (nt.1315)	A to G	Ile 105 Val	Lin *et al*., 2003
Exon 6 (nt.2205)	C to T	Ala 114 Val	Lin *et al*., 2003
Exon 7 (nt.2494)	C to G	Phe 151 Leu	Lin *et al*., 2003
Exon 7 (nt.2596)	T to C	silent mutation at codon 185	Lin *et al*., 2003
3' UTR (nt.2681)	G to C	-	Lin *et al*., 2003
5' UTR	TAAAA-repeat deletion	-	Smith *et al*., 1995; Millar *et al.*, 2000
Intron 1	G-insertion	-	Lo *et al*., 1997

* Nucleotide in parenthesis is numbered according to the genomic sequence.

Table 5 Grouping of GSTP variants (modified from Ali-Osman *et al*., 1997; Heenan *et al*., 1998)

GSTP variant	Codon 105	Codon 114
GSTP1*A	Ile	Ala
GSTP1*B	Val	Ala
GSTP1*C	Val	Val
GSTP1*D	Ile	Val

6.2 Catalytic efficiency of GSTP variants

The GSTP1*B (Val105, Ala114) and GSTP1*C (Val105, Val114) variants appeared to have the catalytic efficiency (k_{cat}/ k_m) for CDNB about 3-4 fold lower than GSTP1*A, the wild type proteins (Ile105, Ala114). In addition, both variants with Val105 exhibit a V_{max} for the carcinogenic (+)-anti-BPDE around 3-4 fold higher than the V_{max} for GSTP1*A (Hu *et al*., 1997; Sunberg *et al*., 1998). However, the mean conjugation activity of the GSTP1*D variant (Ile105, Val114) was not significantly low. It implied that the presence of a valine at position 114 of GSTP1*D had little impact on enzyme activity (Heenan *et al*., 1998). This may be explained by the enzyme structure that the residue 114 is located at the beginning of helix 5 outside the H-site, while residue 105 is situated in the central part of the H-site that is responsible for binding of electrophilic substrates (Johansson *et al*., 1998).

The presence of Val in position 105 possibly changes an affinity of the substrate and, consequently, an enzyme activity. These functional differences may result from the change of hydrophobicity, protein-folding or stability in the H-site of the GSTP protein (Johansson *et al*., 1998; Zimminiak *et al*., 1994). It indicates that a change of amino acid residue in position 105 has an effect on the enzyme catalysis, which depends upon the nature of the electrophilic substrate. Besides a direct effect through an interaction with the substrate and an influence on the H-site structure (which is expected to affect substrate affinity), a change of the residue 105 may cause a secondary effect that alters the catalytic activity of the enzyme. Furthermore, changes in volume and hydrophobicity of the amino acid residue 105 was shown to affect the thermal stability of the enzyme. These findings suggested that the expression of different allelic variant of GSTP makes the detoxification properties of a cell change.

Zimminiak and co-workers (1994) used *Escherichia coli* expression system to compare the properties of Ile105 and Val105 enzymes and also worked on a molecular modeling. They reported that the structural change of each enzyme in folding or stability is unlikely. The bulkier Ile residue could result in a smaller, more restrictive H-site. It could be speculated that the higher affinity for CDNB of Ile105

enzyme compared to Val105 variant may be due to a better fit of the relatively small substrate into the H-site of the Ile105 enzyme. Thus, the difference in catalytic efficiency between these allelic variants would depend on the nature of the toxic agent and the substrate selection of the enzyme.

6.3 Genotype and gene (allele) frequencies of GSTP in different population

The frequency of GSTP allelic variants in different population has been reported world-wide. Most studies were focused on the genotype frequencies among individuals with or without cancer. The frequencies of the polymorphisms at positions 105 and 114 of the *GSTP* gene in normal individuals were summarized in Tables 6 and 7. The frequency of homozygous Val105 genotype varied from 5% to 16% in Caucasians, 5% in Aboriginal Australians, 19% in African- Americans and 1% to 5% in Asians. This may suggest that Val105 allele is more common in Caucasians and African-Americans but less common in Asians. It is important to note that, regarding to the frequency of the Val105 homozygote, the Aboriginal-Australians and the Asians may have the same descendant. Interestingly, homozygous Val114 genotype was not found in all population reported. In addition, heterozygous Ala114Val allele is less common than the heterozygous Ile105Val allele.

Table 6 Genotype and gene frequencies of GSTP Ile105Val in different population

Population	N	Genotype frequency			Gene frequency		Reference
		Ile/Ile	Ile/Val	Val/Val	Ile	Val	
Caucasian-Australian	199	0.40	0.51	0.09	0.66	0.34	Harris *et al.*, 1998
Caucasian-American	287	0.42	0.51	0.07	0.67	0.33	Watson *et al.*, 1998
Estonian	202	0.43	0.44	0.13	0.65	0.35	Juronen *et al.*, 2000
Spanish	220	0.50	0.39	0.11	0.70	0.30	To-Figueras *et al.*, 2002
Finish	112	0.55	0.38	0.07	0.74	0.26	Mitrunen *et al.*, 2001
	259	0.53	0.38	0.09	0.72	0.28	Saarikoski *et al.*, 1998
German	64	0.50	0.34	0.16	0.67	0.33	Stanulta *et al.*, 2000
Netherlander	247	0.59	0.36	0.05	0.77	0.23	van Lieshout *et al.*,1999
Polish	170	0.49	0.44	0.07	0.71	0.29	Butkiewicz *et al.*, 2000
British	155	0.51	0.43	0.06	0.73	0.27	Harries *et al.*,1997
Brazilian	591	0.50	0.38	0.12	0.69	0.31	Rossini *et al.*, 2002
	221	0.45	0.47	0.08	0.69	0.31	Canelle *et al.*, 2004
Turkish	265	0.51	0.37	0.12	0.69	0.31	Aynacioglu *et al.*, 2003
Chinese	49	0.65	0.31	0.04	0.81	0.19	Harris *et al.*, 1998
	119	0.71	0.28	0.01	0.85	0.15	Wang *et al.*, 2003
	164	0.69	0.29	0.02	0.84	0.16	Morita *et al.*, 1998
	419	0.71	0.27	0.02	0.84	0.16	Setiawan *et al.*, 2001
	116	0.67	0.30	0.03	0.82	0.18	Watson *et al.*, 1998
Japanese	122	0.76	0.20	0.04	0.86	0.14	Katoh *et al.*, 1999
Korean	110	0.67	0.30	0.03	0.82	0.18	Pae *et al.*, 2003
Indian	40	0.50	0.45	0.05	0.73	0.27	Harris *et al.*, 1998
Aboriginal-Australian	45	0.82	0.13	0.05	0.89	0.11	Harris *et al.*, 1998
African-American	137	0.35	0.46	0.19	0.58	0.42	Watson *et al.*, 1998

Table 7 Genotype and gene frequencies of GSTP Ala114Val in different population

Population	**N**	**Genotype frequency**			**Gene frequency**		**Reference**
		Ala/Ala	Ala/Val	Val/Val	Ala	Val	
Caucasian-Australian	199	0.85	0.15	0	0.93	0.07	Harris *et al*., 1998
Caucasian-American	112	0.95	0.05	0	0.97	0.03	Watson *et al*., 1998
Estonian	202	0.79	0.21	0	0.89	0.11	Juronen *et al*., 2000
Finish	293	0.83	0.17	0	0.91	0.09	Saarikoski *et al*., 1998
German	64	0.75	0.25	0	0.88	0.12	Stanulta *et al.*, 2000
Chinese	50	0.98	0.02	0	0.99	0.01	Harris *et al.*, 1998
Indian	40	0.87	0.13	0	0.97	0.03	Harris *et al.*, 1998
Aboriginal-Australian	52	1.00	0	0	1.00	0	Harris *et al.*, 1998
African-American	114	0.95	0.05	0	0.97	0.03	Watson *et al.*, 1998

6.4 Role of GSTP polymorphism in individual susceptibility to diseases

Individuals with GSTP genotype that decreases detoxifying activity were assumed to have risk when they expose to elevated level of either carcinogen or toxic chemical comparing to those who have the genotype with normal detoxifying activity (Board *et al*., 1981; Warholrm *et al*., 1994). Thus, an association study was raised as a powerful approach to identify genetic variants that affect individual susceptibility to a common disease (Lokmueller *et al*., 2003). The difference in genotype and/or gene frequency in different racial groups indicates that Val105 allele more associates with some diseases than Val114 allele. Many epidemiological studies provided evidences that link the Val105 homozygotes with increase risks for several cancers (e.g. lung, bladder, esophageal, oral and pharyngeal caners) and non-cancer diseases (e.g. glaucoma, leukemia and Parkinson's disease). In contrast, some studies showed no association between the GSTP polymorphism and the following diseases: lung, prostate, breast cancers and other non-cancer diseases as shown in Table 8.

6.5 Role of GSTP polymorphism and gastrointestinal diseases

Although there was inadequate information on the relationship between GSTP genotype and gastrointestinal diseases, some debates on the association have recently been reported (summarized in Table 9). Two independent groups showed that the heterozygous Ile105Val and homozygous Val105 genotypes were associated with oral cancer (Katoh *et al*., 1999; Park *et al*., 1999). However, some studies showed no association between Val105 homozygote and esophageal, gastric and colorectal cancers (Jain *et al*., 2006; Katoh *et al*., 1999; Ates *et al*., 2005; Mark *et al*., 2004; Setiawan *et al*., 2001). In contrast, Martinez and co-workers (2006) suggested that the homozygous Val105 genotype may protect ones from gastric and colorectal cancers. Ates and colleagues (2005) found that the polymorphism at codon 105 of the GSTP in combination with the null genotype of GSTM and GSTT gave rise to a higher risk for colorectal cancer. In addition, Jain *et al*. (2006) demonstrated that an interaction of smoking and alcohol intake with GSTP polymorphism moderately enchanced the risk of esophageal cancers. So, it might be concluded that there were some other factors playing roles along with GSTP genotype in modifying susceptibility to gastrointestinal diseases. Presently, GSTP polymorphism and *H. pylori* infection was only studied in patients with gastric cancer and gastritis (Setiawan *et al.*, 2001) and there is no report on the association of the GSTP polymorphism and the gastric ulcer disease.

Table 8 Association of GSTP Ile105Val polymorphism and individual susceptibility to some diseases

	Disease	Disease association	Reference
Cancer	Head and neck squamous cell carcinoma	N	Morita *et al*., 1999
	Oral cancer	Y	Park *et al*., 1999; Jourenkova *et al*., 1999
	Esophageal cancer	Y	van Lieshout *et al*., 1999
		N	Morita *et al*., 1998; Lin *et al*., 1998
	Pharyngeal cancer	Y	Jourenkova *et al*., 1999
	Laryngeal cancer	N	Jourenkova *et al*., 1999
	Lung cancer	Y	Wang *et al*, 2003; Stucker *et al*., 2002; Ryberg *et al*., 1997; Miller *et al*., 2003
		N	To-Figureras *et al*., 1999; Sarrikoski *et al*., 1998
	Breast cancer	Y	Helzlsouer *et al*., 1998
		N	Wodegiorgis *et al*., 2002
	Gastric cancer	N	Setiawan *et al*., 2001; Katoh *et al*.,1999
	Prostate cancer	Y	Harries *et al*., 1997
		N	Shepard *et al*., 2000
	Bladder cancer	Y	Harries *et al*., 1997
	Testicular cancer	Y	Harries *et al*., 1997
	Colorectal cancer	Y	Stoehlmacher *et al*., 2002
		N	Welfare *et al*., 1999
	Leukemia (AML)	Y	Allan *et al*., 2001
	Leukemia (ALL)	N	Canalle *et al*., 2004

Table 8 (Continue)

	Disease	Disease association	Reference
Non-cancer diseases	Asthma	Y	Fryer *et al*., 2000
	Asthma	N	Adra *et al*., 1999; Hakonarson *et al*., 2001; Aynacioglu *et al*., 2004
	COPD	N	Yim *et al*., 2002; Ishii *et al*., 1999
	Bronchopulmonary dysplasia	N	Manar *et al*., 2004
	Cystic fibrosis	Y	Henrion-caude *et al*., 2003
	Parkinson's disease (pesticides)	Y	Menegon *et al*., 1998
	Alzheimer's disease	Y	Zuntar *et al*., 2004
	Neuroblastoma	N	Bellincampi *et al*., 2001
	Glaucoma	N	Juronen *et al*., 2000
	Cataract	Y	Juronen *et al*., 2000
	Schizophrenia	N	Pae *et al.,* 2003

Y, N = associated and not associated, respectively

Table 9 GSTP polymorphism and gastrointestinal diseases

Gastrointestinal disease	Disease association	Other risk factor	Reference
Oral cancer	Y	Slightly smoking	Park *et al*., 1999
		-	Katoh *et al*., 1999
Esophageal cancer	Y	Smoking and alcohol intake	Jain *et al*., 2006
Gastric cancer	N	-	Martinez *et al*., 2006
		-	Mark *et al*., 2004; Setiawan *et al*., 2001
Gastritis	N	-	Setiawan *et al*., 2001
Colorectal cancer	Y	GSTT null and GSTM null genotype	Ates *et al*., 2005
	N	-	Katoh *et al*., 1999
		-	Martinez *et al*., 2006

Y, N = associated and not associated, respectively