# CHAPTER 2

# REVIEW OF THE LITERATURE

## 2.1 Cytochromes P450 (CYPs) enzymes

The Cytochromes P450 (CYPs) are a constitutive and inducible heam-containing enzymes with an important role in the oxidative, peroxidative, and reductive metabolisms of a diverse range of compounds, including endobiotics, such as steroids, fatty acids, leukotrines, prostaglandins, and bile acids, and xenobiotics, including most of the clinically used drugs and environmental chemical carcinogens (Wrighton and Stevens, 1992; Gonzalez and Gelboin, 1994; Nelson et al., 1996). The term "cytochrome P450" first appeared in literature in 1962 (Omura and Sato, 1962) to describe an unknown pigment in cells which, when reduced and bound with carbon monoxide, produced a characteristic optical absorption peak at 450 nm. It was a microsomal membrane-bound hemoprotein without known physiological functions, which was originally reported as the spectrum of a novel "microsomal carbon monoxide-binding pigment" in 1958 by Klingenberg. Cytochrome P450 is now regarded as the collective name of a large family of hemoproteins, "cytochrome P450 superfamily," which seems to have diversified from a single ancestral protein to many forms during the course of biological evolution and is distributed widely among various forms of life from animals and plants to fungi and bacteria. Cytochrome P450 of all eukaryotes are membrane-bound enzymes, mostly localized to the endoplasmic reticulum and less in the mitochondrial inner membranes.

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To date, more than 400 distinct cytochrome P450 genes have been identified in animals, plants, bacteria and yeast. Multicellular eukaryotic organisms including animals and plants have about 100 or more P450 genes in their genomes, and those many P450 genes are expressed tissue specifically and developmental stage specifically, indicating their diverse physiological functions. (Omura, 1999). Humans have been estimated to have at least 57 putatively functional cytochrome P450 genes and 58 pseudogenes (Dr. Nelson hompage: http//www.drnelson.utmem.edu/ Cytochromep450.html). To aid in communication, a standardized curated nomenclature has been established (Nelson et al., 1996). This nomenclature is based on evolution of the protein sequences, with similar sequences being clustered into families and subfamilies. Enzymes within a family display greater than 40% amino acid sequence identical (e.g. *CYP2A6* and *CYP2B6*), and those within the same subfamily display >55% identical (e.g. *CYP2A6*, *CYP2A7* and *CYP2A13*) (Nelson et al., 1996). The cytochrome P450 is named with the root symbol *CYP*. Families are designated by an arabic number followed by a letter denoting the subfamily, and a further arabic numeral designating the individual gene (Nelson et al. 1993; Nebert et al. 1991). The first cytochrome P450 enzyme named was *CYP1A1*. The italicized names refer to genes, e.g. *CYP2A13*. Thus, *CYP2A13* represents Cytochrome P450 gene 13 in family 2 and subfamily A. Currently, there are 18 different families and 43 subfamilies known in humans (Table 1). *CYP* families 1, 2, and 3 comprise about half of the total number of *CYP*s in mammals and are generally designated as drug detoxification enzymes due to their considerable abilities to metabolize xenobiotic compounds. But they also possess the capacity to metabolize endobiotics. Family 4 members are better known for fatty acid ω- and (ω-1)-oxidation, rather than

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xenobiotic metabolism. Members of families 5–51 (14 families) perform specific physiological functions involving metabolism of specific endobiotics (Schenkman et al., 2003; Choudhary et al., 2004).

The CYP proteins are predominantly expressed in the liver, although specific isozymes are also expressed in many extrahepatic tissues on a smaller scale, including lung, kidney and gastrointestinal tract (Raunio *et al*., 1995). A few CYP forms participating in the metabolism of foreign compounds are found only in extrahepatic tissues. Expression of specific CYP isozymes is often restricted to a particular cell type, and can therefore result in tissue and cell-specific toxicities as a consequence of CYPs-mediated activation of toxic or mutagenic substrates. Individual CYP isozymes have distinct substrate specificities, active site structures and mechanisms of regulation (Gonzalez 1990).

The crucial function of CYPs is the monooxygenation of various substrates. In order to function, both microsomal and mitochondrial CYPs require an electron transfer chain and utilize reduced nicotinamide dinucleotide phosphate (NADPH) as the electron donor of the monooxygenation reactions (Figure 1). Usually the outcome of CYPs metabolism is inactivation of the substrate, however, in some cases the consequence of CYPs metabolism is activation to a reactive intermediate and this can lead to either acute or chronic cellular toxicity (Gonzalez and Gelboin, 1994).

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**Table 1** Human cytochrome P450 families and their main functions.

| CYP family | Main function |
|---|---|
| *CYP1* (3 subfamilies, 3 genes, 1 pseudogene) | Xenobiotic metabolism |
| *CYP2* (13 subfamilies, 16 genes, 16 pseudogenes) | Xenobiotic and Arachidonic acid metabolism |
| *CYP3* (1 subfamily, 4 genes, 2 pseudogenes) | Xenobiotic and steroid metabolism |
| *CYP4* (5 subfamilies, 11 genes, 10 pseudogenes) | Arachidonic acid or fatty acid metabolism |
| *CYP5* (1 subfamily, 1 gene) | Thromboxane A2 synthase |
| *CYP7A* (1 subfamily member) | Bile acid biosynthesis 7-alpha hydroxylase of steroid nucleus |
| *CYP7B* (1 subfamily member) | Brain specific form of 7-alpha hydroxylase |
| *CYP8A* (1 subfamily member) | prostacyclin synthase |
| *CYP8B* (1 subfamily member) | bile acid biosynthesis |
| *CYP11* (2 subfamilies, 3 genes) | steroid biosynthesis |
| *CYP17* (1 subfamily, 1 gene) | steroid biosynthesis and 17-alpha hydroxylase |
| *CYP19* (1 subfamily, 1 gene) aromatase forms estrogen | steroid biosynthesis |
| *CYP20* (1 subfamily, 1 gene) | Unknown function |
| *CYP21* (1 subfamily, 1 gene, 1 pseudogene) | steroid biosynthesis |
| *CYP24* (1 subfamily, 1 gene) | vitamin D degradation |
| *CYP26A* (1 subfamily member) | retinoic acid hydroxylase important in development |

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**Table 1** Human cytochrome P450 families and their main functions (Continued)

| CYP family | Main function |
|---|---|
| *CYP26B* (1 subfamily member) | probable retinoic acid hydroxylase |
| *CYP26C* (1 subfamily member) | probabvle retinoic acid hydroxylase |
| *CYP27A* (1 subfamily member) | bile acid biosynthesis |
| *CYP27B* (1 subfamily member) | Vitamin D3 1-alpha hydroxylase activates vitamin D3 |
| *CYP27C* (1 subfamily member) | Unknown function |
| *CYP39* (1 subfamily member) | 7 alpha hydroxylation of 24 hydroxy cholesterol |
| *CYP46* (1 subfamily member) | cholesterol 24-hydroxylase |
| *CYP51* (1 subfamily, 1 gene, 3 pseudogenes) | cholesterol biosynthesis, lanosterol 14-alpha demethylase |

Data adapted from David Nelson, 2003 (http//www.drnelson.utmem.edu/ Cytochromep450.html)

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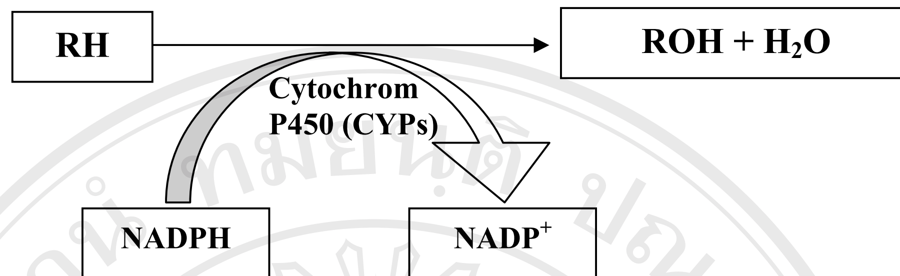


**Figure 1** Representative reaction catalyzed by CYPs. RH represents a broad range of classes of chemical compounds. The P450 catalyzed reaction results in the incorporation of oxygen into the substrate. The outcome of the P450-catalyzed reaction is usually deactivation, but in some cases activation of the substrate to a reactive intermediate can occur.

**2.1.1 Xenobiotic-metabolizing CYPs**

There are two broad groups of human CYPs, a large group whose primary role is the metabolism of xenobiotics (subfamilies 1-4) that are located in the endoplasmic reticulum. The much smaller group of CYPs which are constitutively expressed and located in mitochondrial inner membranes of endocrine glands, where they are specifically involved in steroid hormone and vitamin $D_3$ biosynthesis (e.g. CYP11, CYP17, CYP19 and CYP21) (Gonzalez, 1992; Capdevila et al., 1999).

Cytochrome P450 enzymes in subfamilies 1-4 are located in the endoplasmic reticulum where, along with their redox partner NADPH-450 reductase, they are primarily active in the metabolism of a wide variety of xenobiotics. Indeed, metabolism by one or more P450 isozyme is often the first line of defense of an organism against the

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toxic or mutagenic effects of administered drugs or environmental pollutants. The CYPs-catalyzed reactions increase substrate hydrophilicity by introducing a reactive centre into the substrate molecule (phase I oxidative metabolism), which can then be further conjugated by reaction with phase 2 enzymes such as the glutathione *S*-transferases (GSTs) or *N*-acetyl transferases (NATs) (Gibson and Skett, 1994). P450-mediated oxidative reactions are primarily detoxification processes, which makes the hydrophobic foreign compounds more polar as the first step of their excretion from the body. However, certain substrates are metabolically activated following P450-catalyzed reactions, resulting in the generation of highly reactive products, which react with proteins and nucleic acids causing cytotoxicity or genotoxicity. As a result, inter-individual variation in P450 expression can have profound toxicological consequences (Tucker, 1994).

It is a well known fact that enzymes belonging to the CYP450 family play a key step in the metabolic activation of chemical carcinogens such as carcinogenic compounds in cigarette smoke (Hecht, 1999; Daly, 2003; Jalas et al, 2005; Wong et al., 2005). Procarcinogen may require metabolic activation to exert toxic effects and enzymatically transformed to a series of metabolites as the exposed organism attempts to convert them to forms that are more readily excreted. The metabolic activation process by the CYP450s leads to the formation of DNA adducts, which are carcinogen metabolites bound covalently to DNA, usually at guanine or adenine (Cloutier et al., 2001; Hecht, 2003; Jalas et al., 2005). If DNA adducts escape cellular repair mechanisms and persist, they may lead to miscoding, resulting in a permanent mutation. Cells with damaged DNA

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may be removed by programmed cell death, or apoptosis. If a permanent mutation occurs in a critical region of an oncogene or tumor suppressor gene, it can lead to activation of the oncogene or deactivation of the tumor suppressor gene. Multiple events of this type lead to aberrant cells with loss of normal growth control and, ultimately, to lung cancer (Hecht, 1998, 2003) as shown in Figure 2.

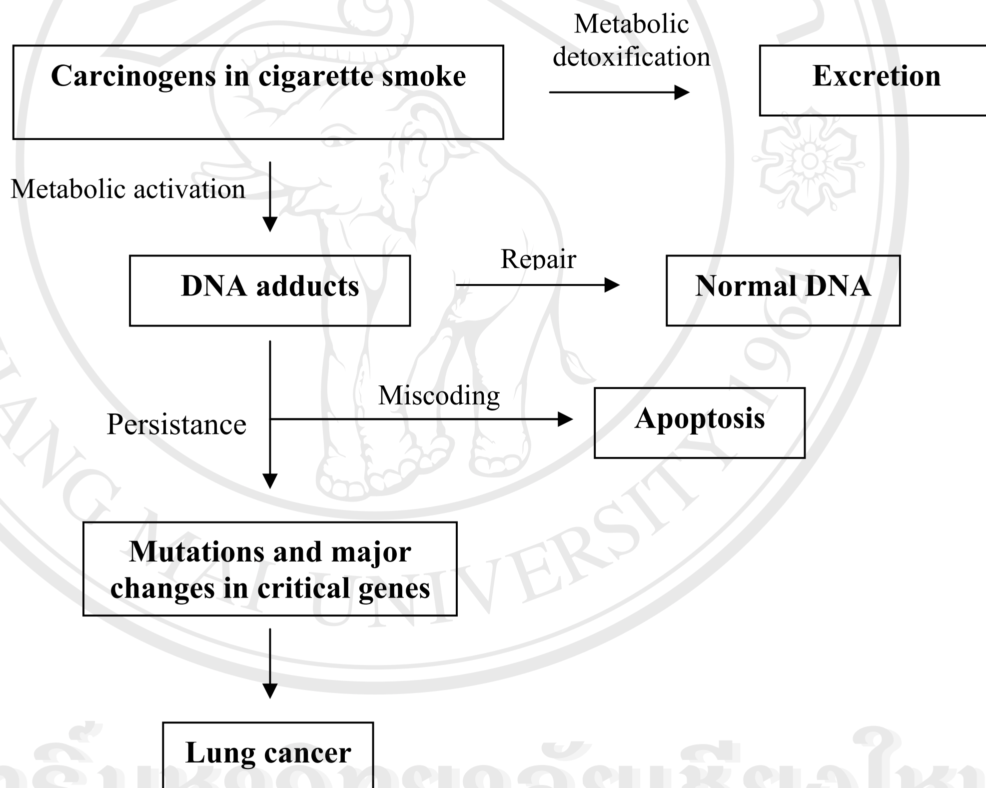


**Figure 2** Schematic linking lung cancer and tobacco smoke carcinogens via metabolic activation and their induction of multiple mutations in critical genes (Modified from Hecht, 1999).

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### 2.1.2 The CYP2A subfamily

The human *CYP2A* genes are located within a region spanning 350-kb on the long arm of chromosome 19 (19q13.2) in a cluster with members of the *CYP2B* and *CYP2F* gene families (Miles *et al*., 1989; Hoffman *et al*., 1995). The human *CYP2A* subfamily comprises three complete genes, designated *CYP2A6*, *CYP2A7,* and *CYP2A13* and two tandemly arranged pseudogenes of *CYP2A7P(T)* and *CYP2A7P(C)* (figure 3). The *CYP2A7* gene is located next to the *CYP2A6* gene. The *CYP2A13* is located next to the two pseudogenes. The human *CYP2A13* gene is the most recently discovered *CYP2A* gene subfamily. The *CYP2A6* and *CYP2A7* genes have a 96% homology in the nucleotide sequence and a 94% identity at the amino acid level (Miles et al., 1989; Yamano et al., 1990). The structure of the *CYP2A* genes consists of 9 exons and 8 introns, and the size of the whole is about 6 kb.

Among the members of *CYP2A* subfamily, two functional genes have been identified in the human *CYP2A* gene subfamily. They are *CYP2A6* and *CYP2A13* (Fernandez-Salguero and Gonzalez, 1995; Su et al., 2000), which share a 93.5% identity in deduced amino acid sequences. The *CYP2A7* is non-functional gene because it encodes an inactive protein that is incapable to incorporate heme (Yamano et al., 1990; Ding et al., 1995). The two pseudogenes, *CYP2A7P(T)* and *CYP2A7P(C)*, are highly homologous to *CYP2A7*, but contain two single nucleotide deletions in exons 3 and 4 which lead to frameshift mutations and the production of truncated versions of *CYP2A7*, lacking a large part of the coding sequence from the 3′ end of exon 5 (Fernandez-Salguero *et al*. 1995).

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As a consequence, these genes do not possess the conserved heme-binding domain and are inactive.

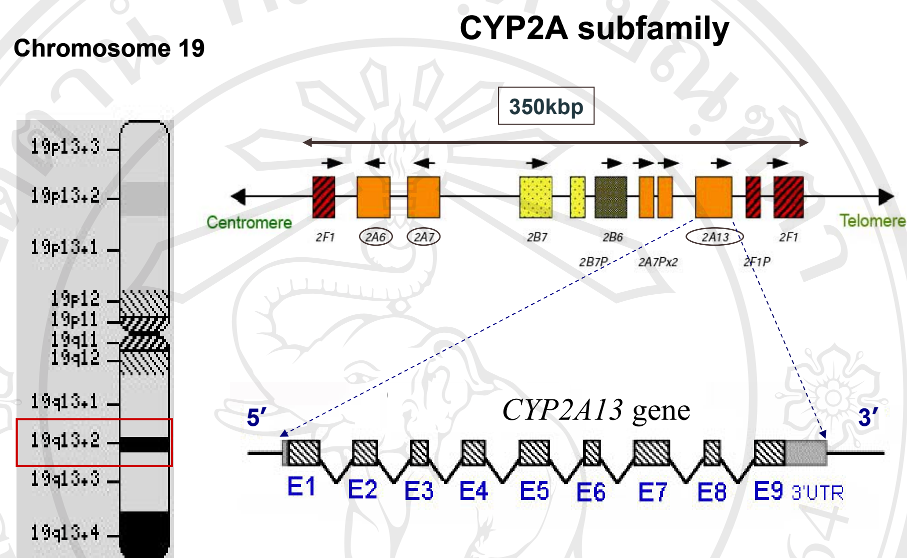


**Figure 3** Structural organization of the *CYP2* family genes on human chromosome 19.A cluster spanning 350 kb is shown The direction of transcription for the different genes are illustrated by arrows. Telomere and centromere directions are also indicated. (Redrawn from Fernandez-Salguero *et al*., 1995).

The expressions of the three *CYP2A* genes were found in the liver and several extrahepatic tissues (Koskela et al., 1999). However, CYP2A6 was more abundant in the liver than CYP2A7 and CYP2A13, which constituting about 4% of total hepatic CYP content (Yun et al., 199; Shimada et al., 1994).

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**2.1.3 Cytochrome P450 2A13 (*CYP2A13*)**

The *CYP2A13* gene is primarily expressed at a high level in the nasal mucosa (Yamano et al., 1990; Miles et al., 1990; Pearce et al., 1992) followed by tracheal mucosa and lung (Su et al., 2000). In all three tissues, *CYP2A13* was expressed at higher levels than was *CYP2A6* (Su et al., 2000; Von Weymarn and Murphy, 2003). In addition, CYP2A13 mRNA was also detected in a number of human tissues, including brain, mammary gland, prostate, testis and uterus, but not in heart, kidney, bone marrow, colon, small intestine, spleen, stomach, thymus, or skeletal muscle (Koskela et al., 1999; Su et al., 2000).

The human CYP2A13 was first identified as the highly active in the metabolic activation of a major tobacco-specific carcinogen, nicotine derived nitrosamine ketone; NNK (4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone), with an α-hydroxylation catalytic efficiency much greater than that of other human cytochrome P450 isoenzyme examined previously (Su et al., 2000). CYP2A13 also catalyzes the α-hydroxylation of other nitrosamines and the metabolism of several nasal toxins such as hexamethyl-phosphoramide, *N*, *N*-dimethylaniline, 2'-methoxyacetophenone, *N*-nitrosomethylphenyl amine, and coumarin7-hydroxylation (Su et al., 2000; Wong et al., 2005). Since lung is the major target organ in NNK-induced carcinogenesis in laboratory animals and in smoking-related human cancers (Hecht, 1998), the high activity of CYP2A13 in NNK α-hydroxylation and its predominant expression in human respiratory tissues suggest that CYP2A13 plays an important role in NNK-induced carcinogenesis.

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**2.1.4 Lung cancer development and NNK metabolism**

The lung is exposed to a wide variety of xenobiotics that may enter the body via inhalation and blood stream and the lung also represents and important target tissue for the toxic effects of exogenous chemicals. As a consequence, the lung is a major target for chemically induced diseases, most notably lung cancer (Hecht 1999). Similarly to liver, human lung has defense mechanisms against substances entering the body. Mechanical, cellular, and enzymatic defense mechanisms act to eliminate hazardous chemicals. In the enzymatic defense reaction, the initial steps are usually carried out by phase I metabolizing enzymes (CYPs) which oxygenate the substrate. The oxygenated intermediates formed in these initial reactions may undergo further transformations by phase II metabolizing enzymes such as Uridine 5'-diphosphate-Glucuronosyltransferases (UGT) Glutathione *S*-Transferases (GST), *N*-Acetyltransferase (NAT), Sulfotransferase (SULT), and other enzymes (Guengerich and Shimada 1991). In general, these enzymatic reactions are beneficial in that they help the pulmonary tissues to eliminate foreign compounds. Sometimes, however, these enzymes transform an otherwise harmless substance into a reactive form (Guengerich 2000).

Cigarette smoking is widely accepted as the major risk factor for the incidence of lung cancer. Multiple carcinogens have been found in cigarette smoke, with polycyclic aromatic hydrocarbons (PAHs), N-nitrosamines, aromatic amines, heterocyclic aromatic amines, and aldehydes forming the major classes of carcinogens (Hecht, 1999). In all, 69 carcinogens in cigarette smoke have been identified that induce lung cancer in animals or humans (Hoffman et al., 2001). Metabolic activation of these carcinogens leads to DNA

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adduct formation. One of the biologically important nitrosamines in tobacco smoke is 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which is believed to play an important role in the induction of cancer in people who use tobacco products. Metabolic actiovation of NNK occurs largely via several cytochrome P450 (CYPs)-catalyzed hydroxylation of the carbon atoms adjacent to the nitroso moiety (i.e. $\alpha$-hydroxylation) (Hecht et al., 1996; Patten et al., 1996; Felicia et al., 2000; Schrader et al., 2000; Su et al., 2000; Fujita and Kamataki, 2001; Jalas et al., 2003)(figure 4). However, there is a significant difference in the catalytic efficiency for NNK $\alpha$-hydroxylation among these P450 enzymes.

To date, the best catalysts of this reaction (i.e. those enzymes with the lowest Km and highest Vmax/Km) are members of the CYP2A subfamily, notably mouse CYP2A5, rat CYP2A3 and human CYP2A13 (Hong et al., 1992; Felicia et al., 2000; Su et al., 2000; Zhang et al., 2002; Jalas et al., 2003). Among all the known human P450 enzymes, CYP2A13 has the highest activity and the lowest *K*m value for NNK $\alpha$-hydroxylation (Su et al., 2000; Jalas et al., 2003). Although CYP2A6, another member of the human CYP2A subfamily, is also active in catalyzing NNK $\alpha$-hydroxylation, its activity to be less than 5% of the CYP2A13 activity (Su et al., 2000).

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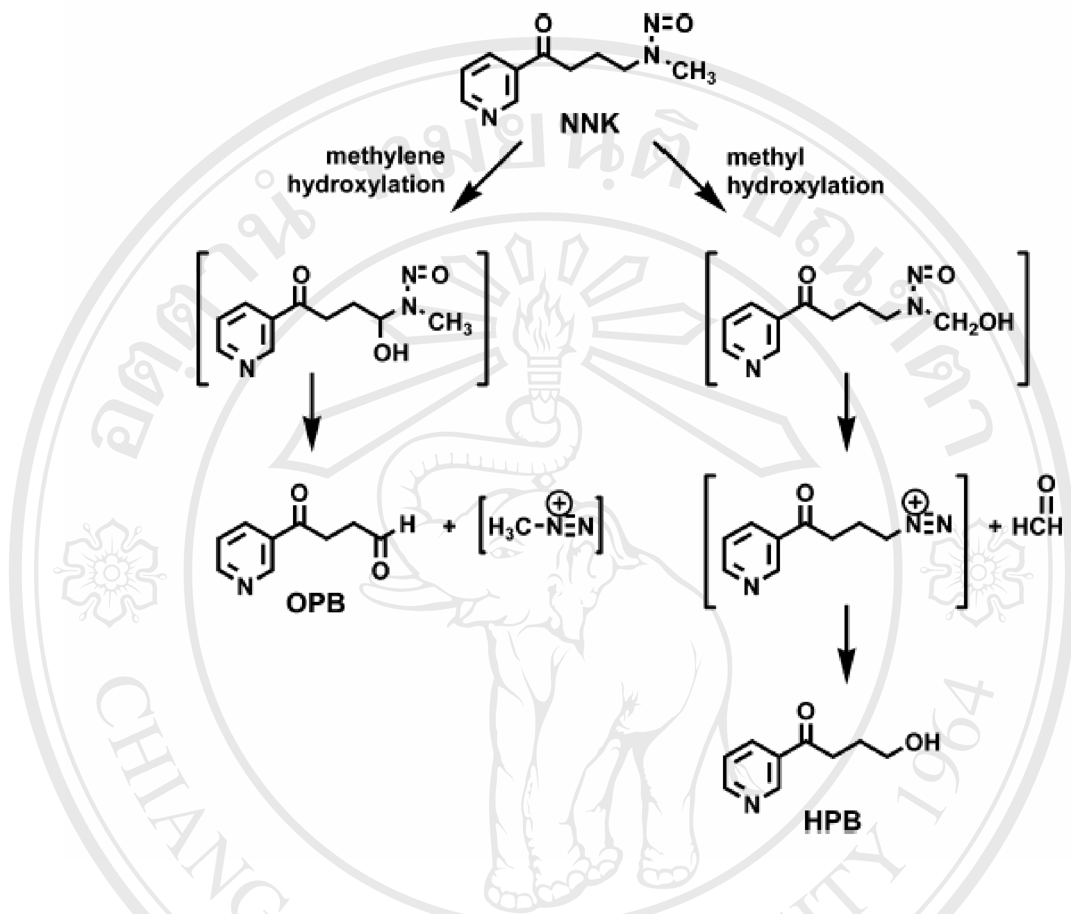


**Figure 4** Representative of α-hydroxylation of NNK via CYPs. α-Hydroxylation is catalyzed by P450 enzymes. Hydroxylation of NNK at the α-methylene carbon results in a DNA methylating agent and 4-(3-pyridyl)-4-oxobutanal (OPB). α-Methyl hydroxylation produces DNA pyridyloxobutylating species and 4-hydroxy-1-(3-pyridyl)-1-butanone (HPB). (Fom: Wong et al., 2005)

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## 2.2 Human Genetic Polymorphisms

Genetic polymorphism is defined as changes in the nucleotide sequence (mutations) which present at least 1% of the population (Pelkonen et al., 1999). The phenomenon has developed during the evolution through various events such as a point mutations or single nucleotide polymorphisms (SNPs) (missense, nonsense or frameshift), gene conversions, deletions, insertions and duplication. SNPs are the most common type of genetic variation in humans that occur when a single nucleotide (A, T, C or G) in the genome sequence is altered. SNPs, which make up about 90% of all human genetic variation, occur approximately once every kilobase along the 3 billion base human genome (Wang et al., 1998). SNPs may occur within coding sequence of gene, non-coding regions of gene, or in the intergenic regions between genes. SNPs within a coding sequence will not necessarily change the amino acid sequence of the protein that is produced, due to redundancy in the genetic code. SNPs that are not in protein coding regions may still have consequences for gene splicing, transcription factor binding, or the sequence of non-coding RNA. Many SNPs have no effect on cell function, but scientists believe others could predispose people to disease or influence their response to a drug (http://en.wikipedia.org/wiki/Single_nucleotide_polymorphism).

Although more than 99% of human DNA sequences are the same across the population, variations in DNA sequence can have a major impact on how humans respond to disease; environmental insults such as bacteria, viruses, toxins and chemicals; and drugs and other therapies. This makes SNPs of great value for biomedical research and for developing pharmaceutical products or medical diagnostics. SNPs are also

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evolutionarily stable, not changing much from generation to generation, making them easier to follow in population studies. Scientists believe SNP maps will help them identify the multiple genes associated with such complex diseases as cancer, diabetes, vascular disease and some forms of mental illness. These associations are difficult to establish with convention gene hunting methods because a single altered gene may make only a small contribution to the disease (http://www.ornl.gov/sci/techresources/Human_Genome/faq/snps.shtml).

### 2.2.1 Polymorphisms of Xenobiotic-metabolizing Enzymes

Genetic variations in the metabolism of various drugs and environmental chemicals have been known for more than four decades (Kalow, 1962). These polymorphisms represent variant alleles that occur at a relatively high frequency in the population and are generally associated with aberrations in enzyme expression or function. Historically, polymorphisms were usually identified following unexpected responses to therapeutic agents. More recently, recombinant DNA technology has enabled scientists to identify the precise alterations in genes that are responsible for some of these polymorphisms. Polymorphisms have now been characterized in many drug-metabolizing enzymes-including both Phase I and Phase II enzymes. As more and more polymorphisms are identified, it is becoming increasingly apparent that each individual may possess a distinct complement of drug-metabolizing enzymes. This diversity might be described as a “metabolic fingerprint”. It is the complex interplay of the various drug- metabolizing enzyme superfamilies within any individual that will

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ultimately determine his or her particular response to a given chemical (Kalow 1962; Nebert 1988; Gonzalez and Nebert 1990; Nebert and Weber 1990).

**2.2.2 Cytochrome P450 2A13 polymorphism**

Several years in the past, the role of *CYP2A13* and the consequences of its genetic variants have become the focus of numerous studies. The *CYP2A13* variants have been identified in different of using PCR-base methods such as Polymerase Chain Reaction coupled with Restriction Fragment Length Polymorphisms (PCR-RFLP), allele-specific PCR, Polymerase Chain Reaction-Single Stranded Conformation Polymorphism (PCR-SSCP) and DNA sequencing.

Recently, a large number of genetic polymorphisms of the *CYP2A13* gene have been identified in several ethnic groups (Zhang et al., 2002; 2003; Fujieda et al., 2003; Cheng et al., 2004). The allele frequency of the 3375C>T (Arg257Cys) polymorphism has been extensively studies and found to be differ among racial groups, with the variant 3375T (Cys257) allele in white, black, Hispanic, and Asian individuals were 1.9%, 14.4%, 5.8%, and 7.7%, respectively. The 3375C>T allele was found most frequently in black (14.4%) and least frequently in white newborns (1.9%). Functional analysis of the variant protein was performed following its heterologous expression. The Arg257Cys variant was 37 to 56% less active than the wild-type Arg-257 protein toward all substrates tested. With NNK, Cys-257 had higher $K_m$ and lower $V_{max}$ values than did Arg257, with a >2-fold decrease in catalytic efficiency. The decreased catalytic efficiency of the corresponding Arg257Cys CYP2A13 protein in NNK bioactivation

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suggests that the Cys-257 genotype may contribute to a lower risk of respiratory tract xenobiotic toxicity. Recently, this polymorphism has been correlated to a reduction in the risk to develop lung adenocarcinoma (OR=0.41; 95% CI=0.23-0.71) in a Chinese population (Wang et al., 2003). The three additional coding region mutations (74G>A, 578C>T, and 1706C>G) and several mutations in the introns and flanking regions were identified in a Chinese population by Zhang et al. (2003). The frequency of the 74G>A (Arg25Gln), 578C>T (Arg101Stop) and 1706C>G (Asp158Glu) missense mutation in Chinese populations were 10.9%, 3.2% and 1.8%, respectively. On the other hand, the 578C>T (Arg101Stop) nonsense mutation was not detected in 136 newborn samples examined (23 white, 21 black, 19 Hispanic, and 73 Asian), suggesting that this mutation may be unique for the Chinese population. Of particular interest, the SNP detected in exon 2 was a 578C>T nonsense mutation, resulting in an amino acid change form an Arg to a stop codon at position 101 which leads to truncated protein which lacking enzymatic activity. Thus, individuals homozygous for this nonsense mutation would not have a functional CYP2A13 protein and, therefore, might have reduced sensitivity to xenobiotic toxicity resulting from CYP2A13-mediated metabolic activation in the respiratory tract. This polymorphism has been associated with the risk of small cell lung cancer (Cauffiez et al, 2004). However, Jiang and his colleagues found no correlation between the 3 most frequent SNPs (74G>A, 3375C>T, and 7233T>G,) and one stop codon (578C>T) mutations with individual susceptibility to nasopharyngeal carcinoma (NPC) in 45 Cantonese NPC patients (Jiang et al., 2004).

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**Table 2** SNP frequency distribution of *CYP2A13* in difference ethnic groups.

| Mutation | Location | Predicted amino acid change | %Frequency (95%CI) | | *n.* | Reference |
|---|---|---|---|---|---|---|
| -1479T>C | 5'-Flanking region | No | Japanese | 13.0 | 192 | Fujieda et al., 2003 |
| -1429A>G | 5'-Flanking region | No | Japanese | 7.3 | 192 | Fujieda et al., 2003 |
| -729C>T | 5'-Flanking region | No | Chinese<br>Japanese | 4.7 (1.2-14.0)<br>4.9 | 32<br>192 | Zhang et al., 2003<br>Fujieda et al., 2003 |
| -12C>A | 5'-Flanking region | No | Japanese | 0.3 | 192 | Fujieda et al., 2003 |
| -411G>A | 5'-Flanking region | No | Chinese | 15.6 (8.2-27.3) | 32 | Zhang et al., 2003 |
| 74G>A | Exon 1 | Arg25Gln | Chinese<br><br>Japanese | 10.9 (4.9-21.8)<br>7.8<br>7.3 | 32<br>709<br>192 | Zhang et al., 2003<br>Jiang et al., 2004<br>Fujieda et al., 2003 |
| 187T>C | Intron 1 | No | Japanese | 0.3 | 192 | Fujieda et al., 2003 |
| 260G>A | Intron 1 | No | Chinese | 4.7 (1.2-14.0) | 32 | Zhang et al., 2003 |
| 281A>G | Intron 1 | No | Chinese | 2.0 | 177 | Jiang et al., 2004 |
| 446T>C | Intron 1 | No | Chinese | 0.6 | 177 | Jiang et al., 2004 |
| 523C>T | Exon 2 | Silent | French | 2.0 | 102 | Cauffiez et al., 2004 |
| 578C>T | Exon 2 | Arg101Stop | Chinese<br>French<br>Tunisian | 3.2 (0.6-12.2)<br>3.8<br>1.0 | 31<br>52<br>48 | Zhang et al., 2003<br>Cauffiez et al., 2005<br>Cauffiez et al., 2005 |

***n.***: number of subjects

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**Table 2** SNP frequency distribution of *CYP2A13* in difference ethnic groups. (continued)

| Mutation | Location | Predicted amino acid change | %Frequency (95%CI) | | *n.* | Reference |
|---|---|---|---|---|---|---|
| 579G>A | Exon 2 | Arg101Gln | Japanese | 0.3 | 192 | Fujieda et al., 2003 |
| 672C>A | Intron 2 | No | Chinese | 4.8 (1.3-14.4) | 31 | Zhang et al., 2003 |
| 1706C>G | Exon 3 | Asp158Glu | Chinese<br>Japanese | 1.8 (0.1-10.8)<br>4.9 | 28<br>192 | Zhang et al., 2003<br>Fujieda et al., 2003 |
| 1757A>G | Intron 3 | No | Chinese | 1.8 (0.1-10.8) | 28 | Zhang et al., 2003 |
| 1801G>C | Intron 3 | No | Japanese | 4.9 | 192 | Fujieda et al., 2003 |
| 1894G>T | Intron 3 | No | Japanese | 0.5 | 192 | Fujieda et al., 2003 |
| 1970A>C | Exon 4 | Silent | Japanese | 4.9 | 192 | Fujieda et al., 2003 |
| 1991C>A | Exon 4 | Silent | French | 1.0 | 102 | Cauffiez et al., 2004 |
| 2211T>C | Intron 4 | No | Japanese | 12.2 | 192 | Fujieda et al., 2003 |
| 3375C>T | Exon 5 | Arg257Cys | Chinese<br><br><br>White<br>Black<br>Hispanic<br>Asian<br>Tunisian<br>Gabonese | 10.9 (4.9-21.8)<br>7.8<br>5.6<br>1.9<br>14.4<br>5.8<br>7.7<br>4.2<br>15.3 | 32<br>709<br>258<br>52<br>52<br>52<br>52<br>48<br>36 | Zhang et al., 2003<br>Jiang et al., 2004<br>Cheng et al., 2004<br>Zhang et al., 2002<br>Zhang et al., 2002<br>Zhang et al., 2002<br>Zhang et al., 2002<br>Cauffiez et al., 2005<br>Cauffiez et al., 2005 |

***n.***: number of subjects

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**Table 2** SNP frequency distribution of *CYP2A13* in difference ethnic groups.(continued)

| Mutation | Location | Predicted amino acid change | Frequency (%) | | n. | Reference |
|---|---|---|---|---|---|---|
| 4947A>G | Intron 5 | No | Chinese | 4.7 (1.2-14.0) | 32 | Zhang et al., 2003 |
| 5294G>T | Exon 6 | No | French | 1.0 | 102 | Cauffiez et al., 2004 |
| 5530T>C | Intron 6 | No | Japanese | 4.9 | 192 | Fujieda et al., 2003 |
| 5578A>C | Intron 6 | No | Chinese<br>Japanese | 4.7 (1.2-14.0)<br>4.9 | 32<br>192 | Zhang et al., 2003<br>Fujieda et al., 2003 |
| 6461T>A | Intron 7 | No | Japanese | 5.2 | 192 | Fujieda et al., 2003 |
| 7196C>T | Intron 8 | No | Japanese | 1.7 | 177 | Jiang et al., 2004 |
| 7233T>G | Intron 8 | No | Chinese | 15.6 (8.2-27.3)<br>10.7 | 32<br>177 | Zhang et al., 2003<br>Jiang et al., 2004 |
| 7343T>A | Exon 9 | Phe453Tyr | Japanese | 0.3 | 192 | Fujieda et al., 2003 |
| 7365T>C | Exon 9 | Silent | French | 1.0 | 102 | Cauffiez et al., 2004 |
| 7465C>T | Exon 9 | Arg494Cys | Japanese | 1.0 | 192 | Fujieda et al., 2003 |
| 7520C>G | 3'-Untranslated region | No | Chinese<br>French<br>Tunisian<br>Gabonese | 4.7 (1.2-14.0)<br>1.0<br>7.3<br>20.8 | 32<br>52<br>48<br>36 | Zhang et al., 2003<br>Cauffiez et al., 2005<br>Cauffiez et al., 2005<br>Cauffiez et al., 2005 |
| 7571G>C | 3'-Untranslated region | No | Chinese | 10.9 (4.9-21.8) | 32 | Zhang et al., 2003 |

***n***.: number of subjects

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