CHAPTER - I
CHAPTER-1

1.1: Introduction

Oral cancer is one of the most common malignancies, and is a major cause of cancer morbidity and mortality worldwide. Approximately 95% of the carcinoma of the oral cavity is squamous cell type in nature. Oral cancer is the 3rd most common cancer in India after cervical and breast cancer [1]. The blemishing effects associated with significant mortality makes the problem more alarming. The most important anatomical bases in the oral cancers are considered the lips, oral cavity and pharynx. The oral cavity includes the front two-thirds of the tongue, upper and lower gums, cheeks and lips lining, mouth floor under the tongue, ‘bony’ top of the mouth and also the small area behind the last teeth.

Till date, there is no consensus as to which site should be included in the assessment of oral cancer. Oral cancer is often regarded as malignant tumors (generally squamous cell carcinoma (SCC)) of the lips, oral cavity (mouth) and the oropharynx [2]. This is usually defined by the following ICD cancer diagnostic groups: intra-oral sites (ICD-10: C00-C06), oropharynx (ICD-10: C09-10), and other ill-defined areas of the lip, oral cavity and pharynx (ICD-10: C14). Other parts include parotid and other major salivary glands (ICD-10: C08-09), and the nasopharynx (ICD-10: C11), pyriform sinus (ICD-10: C12).
Gene polymorphism and risk of oral cancer in a South Indian population – a case control study

and hypopharynx (ICD-10: C13). Here ICD-10 refers to the International Classification of Diseases Coding System of World Health Organization, tenth revision [3]. In order to avoid the confusion caused by different inclusion criteria, the present study concentrated on intra-oral and oral cavity cancers (ICD-10: C01-C06).

One of the real dangers of this cancer is that in its early stages, it can go unnoticed since the onset of oral cancer is painless, and little in the way of physical changes may be obvious. However, the dentist or doctor can, in most cases, see or feel the precursor tissue changes or the actual cancer while it is still very small, or in its earliest stages. In brief, the most common clinical presentation of oral cancer: (i) a red spot, (ii) white and red patch, (iii) white spot, (iv) ulceration or erosion, (v) hardening, (vi) Fixation the surrounding tissue, (vii) lymphadenopathy, (viii), or a combination of these characteristics [4]. The role of potentially malignant lesions has been reviewed by Thompson et al. [5]. In summary, it appears that may arise while the majority of oral cancer de novo, many of the potentially malignant lesions can already show or squamous cell carcinoma in situ, or severe dysplasia reviewed by Gale et al. [6]. However, some carcinomas defined by potentially malignant conditions like: (i) leukoplakia - a supporter of white spot, not diagnosed as any other disease may be, (ii) erythroplakia - a "velvety" red spot not diagnosed
as any other disease, (iii) are speckled leukoplakia - white spots with a red component, (iv) oral submucous fibroid are also found to be associated with oral cancer [7].

1.2: Histopathology

Squamous cell carcinoma is the most frequent (85 to 90%) histological type of oral cancer [8, 9]. Squamous cell carcinomas are often considered to be a single class of lesions associated with relatively benign outcomes and a low risk of metastasis. However, these lesions can show dramatic histopathological diversity and are associated with a wide diversity of clinical outcomes [10]. The histopathological characteristics of the SCC include: invasion of the deeper underlying tissue, varying degrees of squamous differentiation, and cellular pleomorphism, increased nuclear staining [11]. Analysis of 40 squamous cell carcinomas of the oral cavity by flowcytometry revealed its tendency to metastasize to regional lymph nodes [12].

Oral squamous cell carcinoma is histologically classified as: well, moderately or poorly differentiated carcinomas [13]. Well differentiated tumors contain ordered stratification and severe keratinization in a "pear formation, moderately differentiated tumor cells, some layering and poorly differentiated tumors are still recognizable as squamous cell carcinomas, but apparently prominent nuclear pleomorphism
and atypical mitosis [14]. This information is an important part of reporting on the pathological oral cancer, although there is limited evidence of an association between the differentiation status and clinical outcome or treatment discussed [15]. Furthermore, poor tumor histological classifications of oral squamous cell carcinoma is significantly associated with positive nodal status, extracapsular spread, perineural invasion of primary tumors, and the probability of developing distant metastasis after treatment [16]. Although SCC is the most prevalent type of malignant oral tumours, each distinct histological type needs to be considered according to the individual prognosis in patient management and treatment.

The patterns of lymphatic spread - one of the most important routes of oral cancer spread - were reviewed by Zhang et al. [17]. As oral cancer spread through the lymphatic system, including the lymph nodes in the submandibular and deep cervical chain is palpable. Tumors of the tongue and the floor of the mouth show a greater tendency towards regional metastases than cancer of the lip. It should be noted that cancer can show ipsilateral, contralateral or bilateral lymphatic spread [18, 19]. The presence of a lymphocytic response may have the prognostic value, as well as the manner of the invasion [20].
1.3: Diagnosis

The most efficient way to manage oral cancer is to merge early diagnosis and appropriate timely treatment. As more than 90% of all oral cancers are squamous cell carcinomas, the bulk of oral cancers will be diagnosed from lesions on the mucosal surfaces. The diagnosis of early lesions depends on a high index of good clinical judgment and willingness to biopsy of doubtful lesions [21]. Biopsy tissue that collected has to go through histopathological procedures and examining cells and tissues to find out the pathological diagnosis. In the recent past, the histological examination of biopsies and "invasive" radiological imaging techniques are the only methods for diagnosis and assessment of the tumor characteristics. Recently, there is a shift towards non-invasive and in situ tissue diagnosis methods [22, 23].

1.4: Geographical variation

A detailed examination of published sources revealed regional differences in the incidence of oral cancer around the world. Oral cancer is the eighth most common malignancy in the world, with more than 400,000 newly diagnosed cases of oral cancer is estimated that in 2002 [24]. The incidence rates of oral cancer are significantly higher in northern France, Switzerland, Slovakia and the Indian subcontinent than those in the United States, other European countries as well as other parts of Asia [25-27]. Furthermore, 60 to 70% new cases of
oral cancer occur each year in developing regions such as Africa, Central and South America, Caribbean, China, Asia and Polynesia. There are wide variations in the age-standardized Incidence rate of lip and oral cancer in men and women with the highest male rates in the Somme and Bas-Rhin region of France (Figure 1.1.1). The age-standardized Incidence rate of lip and oral cancer in women reported the highest rate in South Karachi, Pakistan, and in Bangalore, India (Figure 1.1.2). Annually, over 300,000 new cases of oral cancer are diagnosed all over the world where the majorities are diagnosed in the advanced stages III or IV. Such data make the oral cancer an important public health matter which is responsible for 3% to 10% of cancer mortality worldwide. There are wide variations in the age-standardized mortality rate of lip and oral cancer in men (Figure 1.1.3) and women (Figure 1.1.4). Like many forms of cancers, oral cancer incidence rate shows the significant increase in incidence with age. This is mainly because of the longer exposure to potential carcinogens and thus increased chances of damage to cellular DNA in the aging cells. In most countries it is reported that about 90% of oral cancer patients are over the age of 40 years, with the average age at diagnosis 65 years [28].
Figure 1.1.1. Age-standardized Incidence rate of oral cancer among men in the countries of the world (Source: GLOBOCAN 2008).
Figure 1.1.2. Age-standardized Incidence rate of oral cancer among women in the countries of the world (Source: GLOBOCAN 2008).
Figure 1.1.3: Age-standardized Mortality rate in oral cavity cancer among men in the countries of the world (Source: GLOBOCAN 2008)
Figure 1.1.4: Age-standardized Mortality rate in oral cavity cancer among women in the countries of the world (Source: GLOBOCAN 2008)
1.5: Etiology and Risk Factors

Smoking and drinking are the main risk factors for oral cancer. Oral cancer risk is linked to both intensity and duration of alcohol and tobacco use. Smoking and alcohol consumption are separate and independent risk factors of oral cancer and alcohol use synergizes with tobacco as a super-multiplicative factor for this disease [29]. Many researchers have found that at least 75-80% of oral cancers to alcohol and tobacco-attributable exposure [30-33].

1.5.1: Tobacco

In fact, smokers are six times more likely to get an oral cancer than nonsmokers. The International Agency for Research on Cancer (IARC) [34] reported that smoking of various forms of tobacco such as cigarettes, bidis, kreteks, cigars and pipes are carcinogenic to humans. Tobacco contains at least 50 known carcinogens, including polycyclic aromatic hydrocarbons, such as tobacco-specific nitrosamines (TSNA) [35-37]. The major tobacco carcinogens include aromatic hydrocarbons such as benzo(a)pyren; the tobacco-specific nitrosamines (TSNs), such as N-nitrosonornicotine (NNN) and 4-(methylnitrosoamino) -1-(3-pyridyl) -1-butaneone (NNK). These compounds are primarily generated by pyrolysis, but are also produced endogenously from smokeless tobacco. Smokeless tobacco products have several carcinogens, these carcinogens are less than those in tobacco smoke [38].
The high incidence of oral cancer in South Asia, particularly in the Indian subcontinent is the use of smokeless tobacco products [39, 40]. There are basically two types of smokeless tobacco: chewing tobacco and snuff. Smokeless tobacco products differ greatly from region to region and culture [41]. In some countries the tobacco chewed is often mixed with some other substances, such as betel quid and areca nut. The areca nut or betel can be chewed alone or as a quid. It is still unclear whether it is the tobacco or the areca products like substance that added plays the major role in the etiology of oral cancer [42-44].

1.5.2: Alcohol

Heavy, regular alcohol consumption is a risk factor for oral cancer. It is estimated that 75 to 80 percent of those with oral cancer drink alcohol frequently. Oral cancer risk significantly increased both the intensity and duration of alcohol use, and it can directly increase with alcohol concentration [45, 46]. Despite this, several studies suggest that all types of alcoholic beverages contribute to oral cancer risk of being responsible with ethanol as a common component [42, 47]. Unpredictably high frequency of oral cancer in certain regions of France was found due to too much consumption of crude distillate [48]. It is not yet proven that alcohol itself is a direct carcinogen, but it may interfere with the development of cancer through different mechanisms. Acetaldehyde, the first metabolite of ethanol is a known carcinogen [49].
1.5.3: Virus infection

Several viruses have been proposed to play a part in the carcinogenesis of oral cancer. However, hardly any study has shown the direct association of these viruses with oral cancer. Infection with human papilloma virus (HPV) is one of the determinants of oral cancer risk groups, particularly those with the lingual and palatine tonsils in the oropharynx [37, 50, 51]. The estimated attributable proportion of oral and oropharyngeal SCC HPV infection is 35% [52]. The degree of oral HPV infection may help to reduce the risk of oral cancer with tobacco or alcohol consumption increase is currently unclear [37, 53].

1.5.4: Diet and nutrition

The strong inverse relation between fruit and vegetable consumption and oral cancer has been demonstrated after adjustments to the effects of tobacco and alcohol. Several studies showed that a diet high in vegetable and fruits, especially rich in micronutrients such as carotenoids, vitamins C, E could provide strong protective effect against oral cancer [54]. Similarly, a cohort study of risk factors for second primary cancers in patients with a history of oral and pharyngeal cancer (OPC), found that eating fruits and vegetables has a protective effect [55]. In another study found, fruit consumption to be protective against oral premalignant lesions [56]. This was supported by studies from India where increased risk
of oral cancer with declining body mass index was observed [57].

1.5.5: Oral hygiene and dental factors

The connection between poor oral hygiene, bad dental status and oral cancer has been noticed. Poor oral health from long-standing dental and gum disease may make carcinogens more potent and more likely to cause oral cancer [58, 59]. For patients of poor oral hygiene, microorganism from dental plaque may contribute to chemical carcinogenesis by the elaboration of toxins such as nitrosating enzymes. Inadequate oral hygiene also fails to dilute carcinogens in the oral cavity. Poor dental status was also proposed as the factor accounting for the elevation in oral cancer risk [60].

1.5.6: Inheritance and genetic background

A survey from Italy revealed that the individuals with a positive family history of oral cancer were significantly more likely to identify risk factors for oral cancer correctly [61]. In cancer, cells divide and grow uncontrollably, forming malignant tumors, and invade nearby parts of the body. The development of oral squamous cell carcinoma (OSCC) is a multistep process requiring the accumulation of multiple genetic alterations, influenced by a patient’s genetic predisposition as well as by environmental influences, including tobacco, alcohol, chronic inflammation, and viral infection [62]. Some
genetic effects were found in oral cancer patients, but the exact procedure is not yet entirely clear. Tumor suppressor genes encode proteins that typically transduce negative growth-regulatory signals and often involved in cell-cycle regulation, including cell-cycle arrest and apoptosis. Inactivation of these genes may be an important step during oral cancer development [63-65]. Oncogenes are genes derived through alteration of cellular proto-oncogenes, which encode proteins that mediate positive cell growth-regulatory and/or cell survival signals. Most of these oncogenes promote aberrant cell proliferation by overriding the G/S, G/M, and M checkpoints of the cell cycle. The role of these oncogenes in the development of oral cancer is well studied [66, 67]. A better understanding of the underlying genetic events of oral cancer suggests promising advances in early detection, risk assessment, diagnosis and prognosis, as well as novel approaches to treatment [68].

1.6: Literature review on Xenobiotic Metabolism

Xenobiotic metabolism is the set of metabolic pathways that modify the chemical structure of xenobiotics, such as environmental components and pharmaceuticals to endogenously produce reactive substances [69, 70]. The liver is one of the most important organs in the body when it comes to detoxifying or getting rid of foreign substances or toxins but a great amount of detoxification occurs in the gastrointestinal
tract as well [71]. The detoxifying enzymes are highly polymorphic exhibiting wide phenotypic variation. Impaired ability to remove reactive substances from the body may lead to chronic disease conditions [69]. Xenobiotic metabolising enzymes are critical components in removing or detoxifying reactive metabolites of xenobiotics which make these enzymes candidates as risk factors [72]. The outcome of biotransformation in most cases is detoxification; nevertheless, metabolism of some xenobiotics produces metabolites that are more reactive than their substrate compound. The biotransformation system involves several enzyme systems that are commonly divided into two phases; phase I and phase II. The phase I enzymes are responsible for oxidation, reduction or hydrolysis and can be either detoxifying or activating [71]. The phase II enzymes except mainly detoxifying potential by conjugation [69].

The cytochrome P450 enzyme superfamily, including CYP1A1 and CYP2E1 constitutes the majority of Phase I enzymes, while the microsomal epoxide hydrolase (mEH) and N-acetyltransferases (NATs) are phase II enzymes primarily responsible for detoxification of xenobiotics. Accumulating data suggest that genetic polymorphisms in genes controlling carcinogen metabolism under individual variations in cancer risk [73-75].
1.7: CYP1A1

The CYP1A1 gene encodes a member of the cytochrome P450 superfamily. The CYP1A1 is a phase I detoxifying enzyme which catalyzes the conversion of environmental procarcinogens to reactive, carcinogenic intermediates. The CYP1A1 protein was found to localize to the endoplasmic reticulum, and it is inducible by polycyclic aromatic hydrocarbons (PAHs) which can be found in cigarette smoke [76] and is involved in the activation of PAHs into reactive epoxide metabolites [77, 78]. The CYP1A1 gene coding for is composed of seven exons and located on 15q22-24 [79]. The second exon starts the open reading frame, encoding a protein of 512 amino acids [80, 81]. CYP1A1 mRNA is expressed in most human tissues, e.g., Lung, esophagus, stomach, small intestine, colon, prostate and breast [82, 83].

Common CYP1A1 polymorphic sites and their nomenclature were well documented table 1.1. Certain polymorphic variants of CYP1A1 gene are known to cause enhanced enzyme activity and play a major role in the pathogenesis of several cancers [84-86]. The first reported CYP1A1 polymorphism (T3801C) was located in the 3’ noncoding region of CYP1A1 and results in CYP1A1*2A allele and can be detected by the MspI restriction enzyme [87]. An additional MspI restriction site in the 3’ non-coding region (T3205C) was identified in African-Americans, resulting in
CYP1A1*3 allele, but this has not been reported among Caucasians or Asians [88]. Several single nucleotide polymorphic sites in the coding regions of the CYP1A1 gene were identified, and many of them are non-synonymous, that is, the variation alters the amino acid sequence of the protein that is produced. Among them, two polymorphisms in exon 7 result in amino acid changes at codons 461 (Thr461Asn) and 462 (Ile462Val), which are situated near the heme-binding region of the protein and modulates the enzymatic activity [89, 90]. These alleles are named CYP1A1*4 and CYP1A1*2C, respectively. Even though these polymorphisms are located in adjacent codons, they are not linked with each other but Ile462Val is in strong linkage disequilibrium with T3801C among Caucasians [91, 92]. Resequencing of exons and flanking intronic regions of CYP1A1 gene in 93 human DNAs representing Caucasian, African-American and Asians revealed eight nonsynonymous SNPs [93]. The Gly45Asp (rs4646422) in CYP1A1 is specific for Asians (5% in Chinese, 10% in Japanese). The Thr461Asn (rs1799814, CYP1A1*4) is specific for Caucasians (6%). The famous and well studied Ile462Val (rs1048943, CYP1A1*2C) in CYP1A1 is present in both Caucasians (7%) and Asians (35%).
**Figure 1.2.1:** Effect of *CYP1A1* and AhR mediated carcinogenesis

Modified and adopted from Singhal et al. 2008 [94].

**Figure 1.2.2:** Schematic representation of human *CYP1A1* gene with studied polymorphisms.

The numbered boxes represent the exons; polymorphisms investigated are indicated by red arrows (see also table 1.1 for nomenclature of alleles).
Table 1.1: Nomenclature of CYP1A1 alleles (taken from http://www.cypalleles.ki.se/cyp1a1.htm).

<table>
<thead>
<tr>
<th>Allele</th>
<th>Protein</th>
<th>Nucleotide changes in Gene</th>
<th>Trivial name</th>
<th>Effect</th>
<th>Enzyme activity</th>
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<td></td>
<td></td>
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<td>Normal</td>
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<td>CYP1A1.2</td>
<td>2454A&gt;G; 3798T&gt;C (MspI)</td>
<td>I462V</td>
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<tr>
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<td>I462V</td>
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1.7.1: **CYP1A1 and cancer proneness**

Various *CYP1A1* gene polymorphisms have been found to be differentially associated with increased risk of several cancers.

1.7.1.1: **Lung cancer and CYP1A1**

Although 90% of all lung cancer patients have a history of smoking, less than 20% of smokers only develop the disease. Even though many studies failed to show a significant association between lung cancer and *CYP1A1* variants, an apparent support exists for the association with at least one of them [100-103]. The 462Val (*2C) is most frequently associated with an increased risk of lung cancer across several populations, also in subjects devoid of tobacco use [100, 104, 105]. The *2A 3801C was first considered to be responsible for the association with lung cancer [106], but is now believed to be in linkage disequilibrium with the 462Val allele in at least some populations [107, 108].

1.7.1.2: **Esophageal cancer and CYP1A1**

Esophageal cancer is mostly associated with excessive intake of alcohol and to some extent related to smoking as well. The association between *CYP1A1* polymorphisms and esophageal cancer risk were performed extensively in Asian individuals, the results were contradictory. However, numerous studies found 2 to 3-fold risk increase among homozygous
CYP1A1*2B carriers [109-111], others could not demonstrate significant association for the same[112, 113] or even found a decreased risk in persons with variant alleles [114]. CYP1A1 Msp1 T/C genotype did not influence the susceptibility of developing esophageal cancer. The Msp1 genotypes along with environmental exposures also did not modulate the cancer risk in North Indians [115]. Meta analysis using the 26 studies suggested that the CYP1A1 exon7 polymorphisms may be a risk factor for esophageal cancer in Asians but not in Caucasians, whereas CYP1A1 Msp1 was not associated with increased susceptibility to esophageal cancer [116].

1.7.1.3: Gastric cancer and CYP1A1

Alcohol intake, smoking, dietary compounds such as a diet high in salty and smoked foods; a diet low in fruits and vegetables are the important risk factors for gastric cancer [117]. The CYP enzymes that involved in the metabolism of these substrates and the polymorphisms in their coding genes are the important modifiers for the associations with gastric cancer. In fact the CYP1A1 gene polymorphisms were not intensively studied in gastric cancer. A Chinese cohort study revealed 50% risk reduction in individuals carrying the *2A variant [118]. CYP1A1 gene polymorphisms in Japanese patients failed to show the association with gastric cancer [119]. A remarkable and statistically significant 36.5-fold increase in the risk of gastric cancer was observed among
patients with \textit{CYP1A1}*2A/*2A combined with GSTM1*0/*0 Lebanese population [120].

1.7.1.4: Colorectal cancer and \textit{CYP1A1}

The exact causes of colorectal cancer are still unknown, but certain risk factors are known to increase the chances of developing this disease. Numerous studies have associated colorectal adenoma with smoking and large bowel cancer with consumption of foods potentially containing polycyclic aromatic hydrocarbons. Eating low amounts of red meat, three servings of vegetables a day and using multivitamins with folic acid have been associated with a lowered risk of CRC. \textit{CYP1A1} gene MspI and Ile462Val mutant genotype was significantly associated with colorectal cancer in Japanese and Hawaiians indicating that the \textit{CYP1A1} is involved in the etiology of colorectal cancer [121]. In Caucasian \textit{CYP1A1}*2 was associated with an increased risk of colorectal cancer in both smokers and non smokers [122, 123]. In Hungarians, the \textit{CYP1A1}*2 was over represented in cases but it is not associated with colorectal cancer [124]. Interestingly, two \textit{CYP1A1} SNPs (Thr461Asn and -1738A>C) showed significant reduction in the risk of cancer [74]. A case control study from Northeast Scotland the \textit{CYP1A1}*4 (m4) variant showed a significant reduction in the risk of cancer but \textit{CYP1A1}*2A (m1) variant is not associated with colorectal cancer [125]. In Brazilian patients the \textit{CYP1A1}*2C (m2) “G” allele was
associated with an increased risk of colorectal cancer but this polymorphism did not show any correlation between sex, grade of differentiation, stage, or evolution of the disease [126]. A recent meta-analysis to study the relation between CYP1A1 Ile462Val polymorphism and colorectal cancer risk revealed that CYP1A1 Ile462Val polymorphism was significantly related with colorectal cancer risk. Subgroup ethnicity analysis showed that CYP1A1 Ile462Val polymorphism was also significantly related with colorectal cancer risk in Europeans and Asians [127]. Another meta-analysis that examined the association between CYP1A1 (MspI and Ile462Val) polymorphisms and risk of colorectal cancer revealed that only Ile462Val polymorphism was associated with risk of colorectal cancer. Ethnic subgroup analyses revealed that significant associations were found in Asians and Caucasians. On the contrary, CYP1A1 MspI polymorphism does not seem capable of modifying colorectal cancer risk indicating that CYP1A1 gene was a low-penetrance susceptibility gene in colorectal cancer development [128].

1.7.1.5: Hepatocellular cancer and CYP1A1

Hepatocellular carcinoma (HCC) is the most common type of liver cancer. Most cases of HCC are secondary to either a viral hepatitis infection or cirrhosis. Therefore, studies on the risk of CYP gene polymorphisms and hepatocellular carcinoma are usually performed in hepatitis-infected patients.
In Taiwanese hepatitis B virus carriers, the \textit{CYP1A1} Mspl or Ile462Val variant alleles increased the risk of HCC among smokers, but in non-smokers [129]. However, a similar study in Italian hepatitis C patients failed to replicate the similar results [130]. This could have been caused by the population stratification in the distribution of Mspl variant allele frequencies [131].

1.7.1.6: Breast cancer and \textit{CYP1A1}

Many studies have shown that postmenopausal women who have the high levels of the estrogen estradiol in their blood have an increased risk of breast cancer. Therefore, CYPs involved in the estrogen pathway are considered as important candidate genes for the susceptibility to breast cancer. In relation to breast cancer risk only few CYP polymorphisms have been identified but these studies did not study the interaction with estrogens [132-134]. The associations between the \textit{CYP1A1} polymorphisms and breast cancer risk have been studied extensively in the various ethnic populations with unconvincing results [92, 133, 135-141].

Individuals possessing \textit{CYP1A1} MSPI variant allele exhibited an increased risk for breast cancer has been documented among African-American, Indians and postmenopausal Chinese women [142-145], whereas a decreased risk in Japanese, Brazilian non-whites [132, 133,
146]. However, later studies which included a larger number of study subjects failed to show statistically significant association between \textit{CYP1A1} genotypes and breast cancer among Chinese, Japanese and African-Americans [139, 141, 147], this is supported by a meta analysis in which the MSPI variant allele is not associated with breast cancer [148, 149]. \textit{CYP1A1} 461Asn allele showed increased risk of breast cancer in Caucasians of French-Canadian origin [150]. Women with \textit{CYP1A1} 462Val variant allele in Caucasian showed statistically significant association with breast cancer [151]. A pooled meta-analysis with >9552 subjects suggests that Val462Val genotype is associated with a trend of reduced breast cancer risk, both in east-Asian women and in pre-menopausal women worldwide [148]. Recent meta-analysis points to the A2455G G allele as a risk factor for breast cancer among Caucasian subjects and did demonstrate significant associations between the MspI, T3205C and Thr461Asn polymorphisms and breast cancer [152].

1.7.1.7: Prostate cancer and \textit{CYP1A1}

Prostate cancer is one of the most common, yet least talked about, hormone-related cancers in men. Therefore, several CYP candidate genes were studied individually as well as in combination. As tobacco-induced CYPs increased risk of prostate cancer [153-155], several studies has investigated the association between \textit{CYP1A1} and prostate cancer. \textit{CYP1A1}
Ile462Val increased risk of prostate cancer in several populations [153, 154, 156, 157]. The CYP1A1 gene polymorphisms are not significantly associated with prostate cancer in Chinese [158], Turkish [159] and Brazilian population [160, 161]. A pooled meta-analysis with 2573 subjects suggests that the CYP1A1 MSP1 polymorphism is likely to increase the risk of sporadic prostate cancer on a wide population basis, the Ile462Val polymorphism may not influence this risk [162].

1.8: CYP2E1

CYP2E1 is a well-conserved xenobiotic-metabolizing CYP enzyme. CYP2E1 is expressed in liver, kidney, nasal mucosa, brain, lung, and other tissues [163]. CYP2E is inducible by ethanol, acetone, and other low-molecular weight substrates. This enzyme is also strongly influenced by nutritional and physiological conditions. It is inducible by high-fat diet, starvation, and diabetes. Many CYP enzymes function in the liver, but the presence of CYP2E1 in the brain is demonstrating its role in both nicotine and ethanol metabolism. A two-to-three-fold increase in CYP2E1 expression in multiple regions of the brain after introducing ethanol or nicotine indicates induction of CYP2E1 upon their administration [164]. The CYPs are regulated not only directly by nicotine and ethanol but also indirectly via an increase in the ethanol consumption in the presence of nicotine pre-treatment [165].
When alcohol consumption is high, the *CYP2E1* catalyzes ethanol into acetaldehyde and produces reactive oxygen species (ROS) and N-nitrosamines [166, 167]. N-nitrosamines are formed endogenously in the stomach and are present in various environmental factors including tobacco smoke. The *CYP2E1* gene is mapped to chromosome 10q24.3-qter. The gene spans over 11 kb and contains 9 exons coding for a membrane-bound protein consisting of 493 amino acid residues with a molecular weight of ~ 57 kDa [168]. Both the 5′-flanking region (5′-FR) and 3′-untranslated-region (3′-UTR) harbour several mutations (Table 1.2) known to alter the transcriptional activity of the gene [169, 170]. Two point mutations in the 5′-FR PstI and RsaI which are in close linkage disequilibrium are known to generate the *CYP2E1_1* (c1) allele and the less common *CYP2E1_2* (c2) allele. PstI and RsaI have been associated with a greater risk for oral, pharyngeal [171, 172], liver [173, 174] and lung cancers [175, 176]. The rare c2 allele frequency constitutes 24–30% for East Asian populations [177, 178], 2–3% for Caucasians [177, 179], 0.3–7% for Afro-Americans [180, 181], 15% for Mexican Americans [180] and 18% for Taiwanese [182].
Figure 1.2.3: Effect of *CYP2E1* and alcohol-mediated carcinogenesis

Modified and adopted from Seitz and Stickel 2007 [166]

Figure 1.2.4: Schematic representation of human *CYP2E1* gene with studied polymorphisms.

The Numbered boxes represent the exons; polymorphisms investigated are indicated by red arrows (see also table 1.2 for nomenclature of alleles).
Table 1.2: Nomenclature of CYP2E1 alleles (taken from http://www.cypalleles.ki.se/cyp2e1.htm).

<table>
<thead>
<tr>
<th>Allele</th>
<th>Protein</th>
<th>Nucleotide changes, Gene</th>
<th>RFLP</th>
<th>Effect</th>
<th>Enzyme activity</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CYP2E1*1A</td>
<td>CYP2E1.1</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
<td>[183]</td>
</tr>
<tr>
<td>CYP2E1*1B</td>
<td>CYP2E1.1</td>
<td>9896C&gt;G</td>
<td>TaqI-</td>
<td></td>
<td></td>
<td>[184, 185]</td>
</tr>
<tr>
<td>CYP2E1*1C</td>
<td>CYP2E1.1</td>
<td>6 repeats in the 5' flanking region</td>
<td></td>
<td></td>
<td></td>
<td>[186]</td>
</tr>
<tr>
<td>CYP2E1*1D</td>
<td>CYP2E1.1</td>
<td>8 repeats in the 5' flanking region</td>
<td>DraI and XbaI</td>
<td></td>
<td>Increase activity after alcohol exposure and in obese subjects</td>
<td>[186, 187]</td>
</tr>
<tr>
<td>CYP2E1*2</td>
<td>CYP2E1.2</td>
<td>1132G&gt;A</td>
<td>R76H</td>
<td>Reduced</td>
<td></td>
<td>[188]</td>
</tr>
<tr>
<td>CYP2E1*3</td>
<td>CYP2E1.3</td>
<td>10023G&gt;A</td>
<td>V389I</td>
<td></td>
<td></td>
<td>[188]</td>
</tr>
<tr>
<td>CYP2E1*4</td>
<td>CYP2E1.4</td>
<td>4768G&gt;A</td>
<td>V179I</td>
<td></td>
<td></td>
<td>[189]</td>
</tr>
<tr>
<td>CYP2E1*5A</td>
<td>CYP2E1.1</td>
<td>-1293G&gt;C; -1053C&gt;T (c1&gt;c2); 7632T&gt;A</td>
<td>PstI+ Rsal-</td>
<td></td>
<td></td>
<td>[169, 190, 191]</td>
</tr>
<tr>
<td>CYP2E1*5B</td>
<td>CYP2E1.1</td>
<td>-1293G&gt;C; -1053C&gt;T (c1&gt;c2)</td>
<td>PstI+ Rsal-</td>
<td></td>
<td></td>
<td>[169, 190]</td>
</tr>
<tr>
<td>CYP2E1*6</td>
<td>CYP2E1.1</td>
<td>7632T&gt;A</td>
<td>Dral-</td>
<td></td>
<td></td>
<td>[191]</td>
</tr>
<tr>
<td>CYP2E1*7A</td>
<td>CYP2E1.1</td>
<td>-333T&gt;A</td>
<td></td>
<td></td>
<td></td>
<td>[189]</td>
</tr>
<tr>
<td>CYP2E1*7B</td>
<td>CYP2E1.1</td>
<td>-71G&gt;T; -333T&gt;A</td>
<td></td>
<td></td>
<td></td>
<td>[189]</td>
</tr>
<tr>
<td>CYP2E1*7C</td>
<td>CYP2E1.1</td>
<td>-333T&gt;A; -352A&gt;G</td>
<td></td>
<td></td>
<td></td>
<td>[189]</td>
</tr>
</tbody>
</table>
1.8.1: CYP2E1 and cancer proneness

It is well known that the CYP2E1 not only increase the blood concentration of acetaldehyde but also may activate these carcinogens more strongly. Activated nitrosamines have been linked to the development of numerous cancers. Various CYP2E1 gene polymorphisms have been found to be differentially associated with increased risk of several cancers.

1.8.1.1: Lung cancer and CYP2E1

Results from studies that evaluated the role of CYP2E1 polymorphisms in relation to lung cancer have been discrepant. Half of the studies in which relations between the CYP2E1*5 (PstI+ Rsal-/DraI-) and CYP2E1*6 (DraI-) alleles and lung cancer were investigated did not find any association at all [177, 192-198]. In the remaining studies the CYP2E1*6 (DraI-) polymorphism was associated with an increased risk of lung carcinoma [199-201]. On the other hand, the CYP2E1*5B (PstI+ Rsal-) polymorphism seems to be associated with a decreased risk of lung cancer [181, 192, 203, 204]. CYP2E1 seems to modify the effects of smoking on lung cancer, even though only a minority of studies considered the relation [180, 202]. A recent meta analysis using 4436 cases and 6385 controls from 26 studies reported a decreased lung cancer risk among subjects carrying c1/c2 and c1/c2+c2/c2 genotypes in the Asian population and on the basis of population control in stratified analysis. The CYP2E1 DraI CC
and CD+CC polymorphisms also showed a protective effect for lung cancer [175]. In the other meta analysis in which 21 published studies involving 9380 subjects of the association between CYP2E1 Rsa I/Pst I polymorphism and lung cancer risk revealed both the c2 allele carriers and homozygote c2/c2 caused significant risks in Asian but not in Caucasians genetic models [203].

1.8.1.2: Esophageal cancer and CYP2E1

The frequency of CYP2E1 c1/c1 genotype was significantly higher in Chinese Kazakh’s patients with esophageal cancer (77.9%) than in control subjects and showed 11-fold increase of esophageal cancer risk [204], the wild-type genotype CYP2E1 (*1/*1) has being associated with a 3 to 5 fold increase of esophageal cancer risk in some other Chinese populations [178, 205]. Tandem repeats in the 5’flanking region of CYP2E1 gene, were associated with an increased risk of esophageal cancer in Japanese [192]. The CYP2E1 variant (*6) was also associated with an increased risk of esophageal cancer in South-African subjects [206]. On the contrary to this CYP2E1*5B and *6 are not associated with esophageal squamous cell carcinoma (ESCC) in Brazilians [207]. A recent meta-analysis comprised of 11 published case-control studies with 1,088 cases and 2,238 controls demonstrates that CYP2E1 Rsa I/Pst I c2 allele may be a
decreased risk factor for developing esophageal cancer among Asians populations [208].

1.8.1.3: Gastric cancer and CYP2E1

A preliminary study in relation to gastric cancer in a Japanese population shows no association between CYP2E1 RsaI and gastric cancer [209]. Analysis of CYP2E1/PstI and CYP2E1/DraI demonstrated the possible involvement of the CYP2E1 polymorphism in smoking-induced gastric cancer development in Koreans [210]. Both the wild-type [119, 211], as well as the CYP2E1*5 variant [212] were mentioned as a risk factor for gastric carcinoma in homozygous individuals, but these associations were not reproduced in another study [120, 213-215].

1.8.1.4: Colorectal cancer and CYP2E1

The combined variant of two polymorphisms in the untranslated region of CYP2E1 on chromosome 10 (*2B), related to increased risk of other cancers as well, was associated with an increased CRC risk among Hungarians [124]. However, this result could not be reproduced among Dutch Caucasians [216]. As functionality is not completely unravelled, these conflicting results are difficult to explain [193]. The CYP2E1 c2/c2 genotype is associated with elevated odds ratio for rectal cancer, but not for colon cancer in a Chinese population [217]. Screening of CYP2E1 RsaI and
96-bp insertion polymorphisms in 685 incident cases of colorectal cancer and 778 community controls revealed that the Rsal c2 allele is associated with a decreased risk of rectal cancer. Individuals having one or two 96-bp insertion alleles showed an increased risk of rectal cancer. Individuals with two 96-bp insertion alleles showed a 2.28-fold increase in colon cancer risk [218]. A novel CYP2E1 locus rs1329149 was found to be significantly associated with CRC risk in Southwestern Chinese [219]. A meta-analysis based on 10 case-control studies involving 4979 colorectal cancer cases and 6012 controls revealed no association between CYP2E1 Rsal/PstI polymorphism and colorectal cancer risk. However, in stratified analysis, Caucasians with c2c2 homozygote appeared to have an increased risk of colorectal cancer [220].

1.8.1.5: Hepatocellular cancer and CYP2E1

Less consistent results were found for the association between CYP2E1 and hepatocellular cancer. The CYP2E1 c1/c1 wild-type genotype significantly increased the risk of developing hepatocellular cancer in cigarette smokers but in those who never smoked [221]. On the contrary to this there was no increased risk of hepatocellular cancer in patients with genotypes c1/c2 and c2/c2 in Korean and Japanese [222]. In Japanese HCC patients when covariates including viremia were selected by using stepwise logistic regression analysis the frequency of CYP2E1 C2 allele significantly higher than
those of controls [223]. These results indicate a variety in allele frequencies in these Asian countries, but other explanations could hold as well.

1.8.1.6: Breast cancer and CYP2E1

The cellular distribution and the level of expression of CYP2E1 assessed by immunohistochemistry, revealed that the CYP2E1 protein is expressed in both tumour and normal breast tissue with an increased expression in breast tumours [224]. The ever drinking women with the CYP2E1 c2 allele containing individuals had an increased risk of developing breast cancer compared to non-drinkers with the CYP2E1 c1/c1 genotype in the Korean population [225]. CYP2E1 PstI genotypes were not significantly different between breast cancer patients and controls living in Sousse on the middle coast of Tunisia [226].

1.8.1.7: Prostate cancer and CYP2E1

Analysis of CYP2E1 gene polymorphisms in the Japanese prostate cancer patients and controls did not show the association between CYP2E1 and Breast cancer susceptibility [154]. Several genetic alterations have been associated with sporadic prostate cancer (PCa). The CYP2E1 Rsal polymorphism was not statistically different between prostate cancer and controls but the Dral polymorphisms, the DD genotype is over-represented in prostate cases when compared with the control group and associated with a twofold
increased risk for the development of prostate cancer Portugal population [227]. The individuals with the CYP2E1 C1/C1 genotype and heavy smoking history showed significantly increased risk for prostate cancer in Chinese [228-230].

1.9: EPHX1 (Microsomal epoxide hydrolase)

Oxidation by one or more of the phase I oxidative enzymes such as the CYP superfamily often results in the formation of a reactive xenobiotic epoxide [231]. The microsomal epoxide hydrolase (mEH) encoded by EPHX1 is a biotransformation enzyme that metabolizes numerous reactive epoxide intermediates to more water-soluble trans-dihydropdiol derivatives [231]. EPHX1 is a smooth endoplasmic reticulum enzyme and is expressed relatively ubiquitously in most tissues and in many species [232, 233]. It is likely that CYP and EPHX1 enzymes cooperate via protein-protein interactions, meaning that a metabolite produced by CYP can be directly transferred to the other enzymes participating in the subsequent metabolism [234, 235]. In certain instances, the initial trans-dihydropdiol metabolites are further activated by CYP to form highly electrophilic and reactive dihydropdiol-epoxides that form covalent adducts with DNA [236].

The EPHX1 gene is located in chromosome 1q42.1 [237]. The gene contains nine exons, eight of which are coded [238]. The translated protein of 455 amino acids is the product of a
single gene [239], although alternatively spliced non-coding regions of exon 1 have been reported [240]. The expression of EPHX1 may vary between the human tissues. The alternative promoters are most likely defined the basis for tissue-specific expression of EPHX1 [241], hence its expression levels are much lower in lymphocytes than in liver and lung [242]. The EPHX1 core promoter region has several putative transcription factor binding sites [243] and also a putative binding site for Nrf2 on the ARE that may be involved in the inducible expression of EPHX1 by xenobiotics [244, 245].

The gene coding for microsomal epoxide hydrolase (EPHX1) exhibits Polymorphism [237]. Among these two single nucleotide polymorphisms (Tyr113His and His139Arg) have been described in the coding region of the EPHX1 gene that produces two protein variants [237]. At codon 113, substitution of tyrosine to histidine, decreased EPHX enzymatic activity by approximately 40% and whereas at codon 139, substitution of histidine to arginine, increased EPHX activity by approximately 25% [246]. Of the synonymous changes, Lys119Lys (SNP; rs2292566), resulting from a G to A substitution, has a minor allele frequency of 0.12 in Caucasians (A allele) [247]. This SNP has not any functional consequences, but the close proximity to Tyr113His polymorphism has been shown to cause erroneous genotyping
Gene polymorphism and risk of oral cancer in a South Indian population – a case control study

results for the Tyr113His locus with conventional PCR-RFLP methods [248-252].

Based on the assumption that the Tyr allele at Tyr113His and the His allele at His139Arg confer normal activity, whereas the His allele at Tyr113His confers low activity and the Arg allele at His139Arg confers high activity, Benhamou et al. [252] classified predicted EPHX1 activity as low, intermediate, or high on the presence or absence of the 2 polymorphisms. Based on this assumption activity pattern of samples in all populations was given in table 1.3. These two EPHX1 polymorphisms were studied extensively for many cancers such as lung cancer [253, 254], orolaryngeal cancer [255, 256], breast cancer [226], squamous cell esophageal cancer [257] and acute lymphoblastic leukemia [258].

**Figure 1.2.5: Schematic representation of human EPHX1 gene with studied polymorphisms.**

The Numbered boxes represent the exons; polymorphisms investigated are indicated by red arrows (see also table 1.3 for nomenclature of alleles).
Table 1.3: Predicted EPHX1 activity classified by Benhamou et al. [249].

<table>
<thead>
<tr>
<th></th>
<th>Y113H</th>
<th>H139R</th>
<th>Combination of Y113H and H139R</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>High activity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>139HR,</td>
<td>113YH/139RR,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>139RR</td>
<td>113YY/139HR,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>113YY/139RR</td>
</tr>
<tr>
<td><strong>Intermediate activity</strong></td>
<td>113YY</td>
<td>139HH</td>
<td>113HH/139RR,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>113YY/139HH,</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>113YH/139HR</td>
</tr>
<tr>
<td><strong>Low activity</strong></td>
<td>113YH,</td>
<td></td>
<td>113HH/139HH,</td>
</tr>
<tr>
<td></td>
<td>113HH</td>
<td></td>
<td>113HH/139HR</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>113YH/139HH</td>
</tr>
</tbody>
</table>

1.9.1: EPHX1 in cancer proneness

1.9.1.1: Lung cancer and EPHX1

The role of EPHX1 polymorphisms in susceptibility to lung cancer has been widely studied but the results are inconsistent [253, 254, 259-262]. The main reasons for the differences in results may be adopting an imperfect methodology for genotyping the exon 3 [248]. However, the earlier findings suggest a decreased risk of lung cancer for His113 slow activity allele homozygotes compared to the wild type Tyr113 allele homozygotes [253, 259]. For exon 4 polymorphism, both increased and decreased risks have been reported. Similarly, studies on the associations between the predicted EPHX1 activity and lung cancer risk have also given
inconsistent results [252, 259]. A systematic review and meta-analysis of 13 case-control studies revealed that EPHX1 exon 3 low-activity genotype (H113H) of was associated with decreased risk while the EPHX1 exon 4 high-activity genotype (R139R) was associated with an increased risk of lung cancer among Caucasians. Moreover, the predicted low EPHX1 activity was associated with a modest decrease of lung cancer risk [263]. A recent comprehensive systematic review and meta-analysis of 84 studies also suggested that the predicted low EPHX1 enzyme activity may have a potential protective effect on tobacco-related carcinogenesis of lung and UADT cancers and this association is influenced by cigarette-smoking status [264].

1.9.1.2: Esophageal cancer and EPHX1

The predicted high mEH activity was seen more frequently in cases than controls and also the high activity genotypes of EPHX1 were significantly increased the individual susceptibility to esophageal adenocarcinoma [114, 265]. EPHX1 Tyr113His polymorphism did not show significant difference in allele distribution of esophageal squamous cell carcinoma (ESCC) patients and controls in a population of North China [266]. A hospital based case control study from Taiwan suggest that the EPHX1 His113His genotype can differentiate the association between smoking, areca chewing, and ESCC [267]. EPHX1 gene exon 3, Tyr113His genotype
was associated with higher risk of ESCC particularly at upper and middle-third anatomical locations of tumor [257]. A large-scale pathway-based candidate gene association study using 1330 single-nucleotide polymorphisms (SNPs) in 354 genes failed to show association between *EPHX1* gene polymorphisms and esophageal cancer in Caucasian [268]. A recent study from a high-incidence region of India, the Patients with the 139Arg/Arg genotype were at significantly higher risk for developing a well-differentiated and moderately-differentiated grade of tumor. In contrast, the 113His/His genotype of exon 3 were a significant protective factor for esophageal cancer in tobacco smokers, betel quid chewers, and alcohol users [269].

1.9.1.3: **Gastric cancer and *EPHX1***

A nested case-control study within the European Prospective Investigation into Cancer and Nutrition revealed that only the homozygous variant CC of Y113H in *EPHX1* was significantly associated with increased gastric cancer risk in ever smokers [270]. In contrast to this, polymorphisms in metabolic genes, their combination and interaction with tobacco smoke and alcohol consumption in an Italian population failed to show such a significant association between *EPHX1* polymorphisms and gastric cancer risk [271]. Recent data from a case-control study in Japan also did not observe an association between SNPs in this block and gastric cancer risk [272].
1.9.1.4: Colorectal cancer and *EPHX1*

Analysis of *EPHX1* gene polymorphisms in relation to risk of colorectal adenoma in two case-control studies nested in the Nurses’ Health Study (NHS) and Health Professionals Follow-up Study (HPFS) cohorts did not show a significant association with overall risk of adenoma. This study also indicates that individuals exposed to > or =25 pack-years smoking were at increased risk of colorectal adenoma and that risk is related to dose of tobacco carcinogens and mEH activity level, but the results were not consistent between men and women [273]. In non-Hispanic Whites that recruited from the National Cancer Institute’s Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial, the *EPHX1* polymorphisms are associated with increased risk of advanced colorectal adenoma, particularly among current and recent smokers [274]. In contrast to this several studies has reported that the *EPHX1* polymorphisms are not associated with sporadic colorectal cancer [74, 216, 275]. Analysis of biotransformation gene polymorphisms suggests that allelic polymorphism of metabolizing enzymes plays an important role in human colorectal carcinogenesis by affecting the metabolism of dietary carcinogens [276, 277]. Meta analysis of seven studies using *EPHX1* gene polymorphisms found a weak suggestion of an antagonistic effect of *EPHX1* exon 3 low or medium metabolizer with smoking on colorectal adenoma risk [278]. In
a case-only analysis, unconditional logistic regression was used to examine the associations between smoking and each SNP and between the two SNPs in nonfamilial colorectal adenoma patients, smokers with any variant allele of *EPHX1* were at increased risk for CRC, as were individuals with any variant allele of *CYP1A1* together with any variant allele of *EPHX1* [279]. A recent study of men and women with colorectal adenomas, HPs, or concurrently with both types of polyps and polyp-free controls receiving a colonoscopy failed to suggest the association between mEH genotype and colorectal polyps, nor were any statistically significant gene-environment interactions [280].

1.9.1.5: Hepatocellular cancer and *EPHX1*

In Chinese population the incidence of hepatocellular carcinoma (HCC) is found to be associated with *EPHX1* H113 allele [281]. EPHX 113HH and 139HH genotypes increased the risk of HCC, but not modify the association between peanut butter consumption indicating the unlikely role of EPHX in aflatoxin metabolism [282, 283]. *EPHX1* 113His/His homozygotes were overrepresented in advanced stages of disease, in particular among HCC patients but these differences were more prominent among men than women. The predicted low enzyme activity was more prevalent among cirrhotic and HCC patients indicated *EPHX1* gene polymorphisms were significantly associated with HCV-related
liver disease severity and HCC risk [284]. *EPHX1*, R139R imposed a risk factor for HCC and chronic hepatitis-infected subjects, the combination of *GSTM1* and T1 genotypes with either of exon 3 or 4 polymorphisms and of *EPHX1* exhibited synergistic associations for HCC development [285]. *EPHX1* gene haplotypes also exhibited sharing of a positive association with HCC risk in India [286].

1.9.1.6: Breast cancer and *EPHX1*

Analysis of *EPHX1* genotypes revealed that carriers of *EPHX1* *3/*3 genotype are over-represented among breast cancer cases than in controls. The carriers of predicted low activity alleles were also exhibited higher risk of breast cancer in comparison with carriers of high *EPHX1* activity, but the results are not significant [287]. On contrary to this a significant decrease in breast cancer risk was associated with the *EPHX1* CC genotype when compared with the TT genotype in a case-control study in an Australian Caucasian population-based sample [288]. Investigation of 11 genes encoding key proteins in biosynthesis, catabolism and detoxification in breast cancer cases and controls from Germany failed to establish the relation between estrogen metabolic pathway gene polymorphisms and breast cancer risk [289]. In a subgroup of premenopausal patients with breast cancer the *EPHX1* homozygous mutant genotype has shown a significant association with the risk of breast carcinoma. The
heterozygous $EPHX1$ genotype was also found to be protective against breast carcinoma in the selected population [226]. The association between $EPHX1$ gene polymorphisms and breast cancer was not observed in Thai women [290].

1.9.1.7: Prostate cancer and $EPHX1$

Initial studies in Israeli prostate cancer patients, the $EPHX1$ His113 allele is seemingly associated with a more advanced, late onset disease [291]. Investigation of the association between prostate cancer and smoking, as well as the main and modifying effects of microsomal epoxide hydrolase His139Arg functional polymorphisms failed to show the main effects of smoking or His139Arg polymorphisms [292]. The $EPHX1$ 139Arg/Arg genotype, decreased adducts levels in both prostate tumor and nontumor prostate cells of Caucasians, but this effect was not found in African Americans [293]. Increased prostate cancer risk was observed with high, compared with no, petroleum oil/petroleum distillate in individuals carrying $EPHX1$ rs17309872 [294].

1.10: NAT2 gene

The $N$-acyltransferases (NAT; E.C.2.3.1.5) are xenobiotic-metabolizing enzymes (XME) that involved in the metabolism of drugs, environmental toxins and aromatic amine carcinogens present in cigarette smoke. $N$-acyltransferases catalyze the transfer of an acetyl group from acetylCoA (Ac-
CoA) to the nitrogen or oxygen atom of arylamines, hydrazines, and their N-hydroxylated metabolites [295]. NAT2 gene (MIM # 243400) codes for the NAT2 proteins that have a variable enzymatic activity or stability, leading to slow or rapid acetylation [296, 297]. The human NAT2 gene spans 9.9 kb and is located on chromosome 8p22. NAT2 consists of a non-coding exon at the 5’ end, separated by a 9 kb intron from an uninterrupted coding region of 873 bp that encodes a 290 amino acid protein. The NAT2 gene is polymorphic and 36 alleles have been described till date. Several of the NAT2* alleles share sequence variations, and not all sequence variations would lead to change in the enzyme activity of the encoding protein (Table 1.4). Early genotyping studies screened for the presence of the C481T, the G590A, the G857A and sometimes the G191A nucleotide changes, all of which was shown to cause a slow acetylation phenotype [298]. A threefold decrease in clearance was reported between fast acetylators and slow acetylators [299]. The frequency of the slow acetylator phenotype varies considerably between ethnic groups [300], and this is due to the different frequencies of the polymorphisms that correspond to the slow acetylator alleles. In Caucasian and African populations, the frequency of the slow acetylation phenotype varies between 40 and 70%, while in Asian populations, such as Japanese, Chinese, Korean, and Thai, it ranges from 10 to 30% [301].
Figure 1.2.6: Schematic representation of human NAT2 gene with studied polymorphisms.

The Numbered boxes represent the exons; polymorphisms investigated are indicated by red arrows (see also table 1.4 for nomenclature of alleles).

Table 1.4: Nomenclature of NAT2 alleles (Taken from http://louisville.edu/medschool/pharmacology/NAT.html).

<table>
<thead>
<tr>
<th>NAT2 Allele</th>
<th>Nucleotide Change (NCBI rs Identifier)</th>
<th>Amino Acid Change</th>
<th>RFLP Enzyme</th>
<th>Phenotype</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAT2*11A</td>
<td>481C&gt;T (rs1799929)</td>
<td>L161L (synonymous)</td>
<td>KpnI</td>
<td>Rapid</td>
<td>[302-305]</td>
</tr>
<tr>
<td>NAT2*6B</td>
<td>590G&gt;A (rs1799930)</td>
<td>Arg197Gln</td>
<td>TaqI</td>
<td>Slow</td>
<td>[302-305]</td>
</tr>
<tr>
<td>NAT2*7A</td>
<td>857G&gt;A (rs1799931)</td>
<td>Gly286Gln</td>
<td>BamHI</td>
<td>Slow</td>
<td>[302-307]</td>
</tr>
<tr>
<td>NAT2*6E</td>
<td>481C&gt;T (rs1799929)</td>
<td>L161L, R197Q</td>
<td>KpnI, TaqI</td>
<td>Slow</td>
<td>[308]</td>
</tr>
<tr>
<td></td>
<td>590G&gt;A (rs1799930)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
1.10.1: NAT2 in cancer proneness

1.10.1.1: Lung cancer and NAT2

Initial studies examining the role of the NAT2 polymorphic phenotype in susceptibility to lung cancer were either negative [309] or showed an insignificant over representation of rapid acetylators [310, 311]. Individuals with a NAT2 rapid acetylator phenotype are thought to have an increased NAT2 metabolic capacity and therefore have a protective role against cancer. Consistent with this hypothesis, NAT2 rapid acetylator genotypes have been associated with a decreased risk of lung cancer, an effect that seems modulated by smoking [251, 312]. In contrast, few studies failed to show association between NAT2 acetylator genotype and lung cancer [73, 313-315]. Never-smoking individuals with NAT2 fast acetylator were more prone to lung cancer in Taiwan women but not in men [316]. NAT2 genotype responsible for slow acetylation (NAT2*5B/*6) was observed significantly more frequently in lung cancer patients than control subjects [317, 318].

A recent meta analysis including 3945 lung cancer cases and 6085 controls from 19 published studies which were selected from 29 articles revealed very little evidence of an association between the NAT2 polymorphism and the risk of lung cancer [319].
1.10.1.2: Esophageal cancer and NAT2

Till date it remains a controversy and no consensus concerning whether there is a true association between esophageal cancer and N-acetylation polymorphism. However, analysis of 71 esophageal squamous cell cancer (ESCC) patients and 329 healthy control subjects from Japan revealed over representation of NAT2 slow acetylator phenotype in esophageal cancer patients than in the controls [320]. NAT2 slow acetylator genotype was not significantly associated with risk of esophageal cancer in studies from North India [321] and Iran [322]. In contrast to this another case control study from Kashmir Valley reported that the NAT2 slow acetylator genotype and haplotypes increased susceptibility to ESCC [323].

1.10.1.3: Gastric cancer and NAT2

Previous studies showed that, individuals with NAT2 rapid acetylators are at increased risk of developing gastric cancer in Europeans [324], and in Koreans [325]. In contrast to this, no correlation between NAT2 polymorphic sites and gastric cancer was reported in USA [326], Poland [327], Japan [119, 328, 329] and Omani Arab population [330]. NAT2 slow acetylator genotype is not directly associated with gastric cancer risk but may be an important modifier of the effects of environmental factors on gastric cancer risk [331]. A meta analysis using 13 studies also showed no significant
association in genotype distribution between gastric cancer and control [332].

1.10.1.4: Colorectal cancer and NAT2

Large-scale molecular epidemiological studies that investigate the relationship between the NAT2 varieties and colorectal cancer (CRC) are inconclusive. Several studies have shown an increased risk of CRC in patients with NAT2 rapid acetylators [333-339]. Other studies, focused more on environmental factors acting through procarcinogenic compounds activated by NAT2, did not confirm an increased risk of cancer in patients with NAT2 rapid acetylators [328, 340-349]. Few studies of meta-analyses failed to support the hypothesis that NAT2 alone is an important risk factor for colon cancer and suggests that NAT2 rapid acetylation status has no specific effect on the risk of developing colon cancer [278, 350].

1.10.1.5: Hepatocellular cancer and NAT2

A significant association between NAT2 genetic polymorphism and hepatocellular cancer (HCC) was observed among chronic hepatitis B virus (HBV) carriers who were smokers but not among the non-smokers [351]. The smokers with a slow acetylation genotype of N-acetyltransferase 2 may be a strong risk for hepatocellular carcinoma in Chinese [352] and Germans [353]. N-acetyltransferase 2 polymorphism is not
related to the risk of advanced alcoholic liver disease in Spanish individuals, but the slow acetylator genotype may predispose the ALD patients to develop HCC [354]. Although there is no association between the susceptibility of HCC and the overall NAT2 genotypes, the individuals with rapid acetylators showed increased risk of HCC [355]. No evidence for a gene-environment interaction in HCC risk for NAT2 genotypes was observed [356, 357].

1.10.1.6: Breast cancer and NAT2

The association between NAT2 acetylator phenotype or genotype and breast cancer was investigated in several studies, but the results are inconsistent. In many studies NAT2 acetylator phenotype was not associated with breast cancer [358-360]. However, the rapid acetylator phenotype was associated with breast cancer risk [361, 362]. Rapid-acetylation status was associated with increased risk of breast cancer both in the whole sample and among postmenopausal women [363]. Conversely, slow acetylators increased risk of breast cancer, in postmenopausal women [364, 365]. In a meta- and pooled analysis including 13 studies, NAT2 was not independently associated with breast cancer risk but smoking was found to be associated with increased risk in NAT2 slow acetylators but not in rapid acetylators [366].
1.10.1.7: Prostate cancer and *NAT2*

Previous studies showed that, the slow *NAT2* genotype has been associated with a lowered prostate cancer (PC) risk while the rapid *NAT2* genotype has been associated with a non-significantly elevated PC risk [367, 368]. In contrast to this *NAT2* slow acetylator genotype showed an important role in determining the risk of developing prostate cancer [369, 370]. No relationship between *NAT2* genotype and prostate cancer was also observed in two studies [371-373].