

Polymorphisms in Drug-metabolizing Genes and Risk of Head and Neck Squamous Cell Carcinoma

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ABSTRACT One kind of defense mechanism against development of cancer involves activities of a series of enzymes that metabolize and excrete potentially toxic compounds and repair subtle mistakes in DNA. Head and neck squamous cell carcinoma (HNSCC) is causally associated with tobacco use alone and also in combination with alcohol consumption. Most of the carcinogens present in tobacco and tobacco smoke are converted into DNA-reactive metabolites by cytochrome P450 enzymes and detoxification of these metabolites are performed by glutathione S-transferases and N-acetyltransferases in humans. Several of these genes display polymorphisms that could modulate enzymatic activities like activation and detoxification of carcinogens. Molecular epidemiological studies, to assess the risk associated with metabolic polymorphisms and HNSCC, have shown that overall effect of common polymorphisms is moderate in terms of penetrance and relative risk. However, it may have a great population impact to assess the risk of cancer caused by tobacco habit. In this review, we have discussed the reported prevalences of these common polymorphisms and risk of HNSCC causally related to tobacco habit in different populations. Although a large number of studies with small sample sizes have been conducted, the results have been inconsistent and therefore inconclusive. So, future studies should be conducted with sufficiently large sample sizes to detect the moderate or even small risks in a population.

INTRODUCTION

A tumor is the result of a series of mutations in genes of a single cell or clones of that cell, which lead to loss of normal function, uncontrolled cell growth and often metastases. Many of these genes have been observed to induce cell proliferation (e.g. ras and myc protooncogenes) and also halt proliferation of DNA-damaged cells (e.g. p53 and APC tumor suppressor genes). Mutations in DNA repair, cell

cycle control, angiogenesis and telomerase production genes are also necessary to pass the mutated gene from mother cell to offspring. The pattern of losses and mutations are complex, although mutation or loss of at least one proto-oncogene and one or more tumor suppressor genes are likely to occur in nearly all tumors (Vogelstein and Kinzler 1998). With the exception of rare familial cancers, which are primarily caused by germline inheritance of a specific mutation, a sporadic cancer acquires mutations as a result of gene-environment interactions. The environmental factors, either external (e.g. tobacco carcinogens, dietary factors, infectious agents) or internal (e.g. hormones) or both are metabolized to produce genotoxic compounds which could react with DNA for adduct formations. If not repaired, these adducts may cause mutation in DNA. Mutations in some of the genes (also called high-risk genes e.g. p53, retinoblastoma) are highly penetrating and have dominant effects but the frequency of these mutations is very low (1/10,000). These traits can confer lifetime cancer risks to the affected individuals, but they explain only a small fraction of cancer incidence. So searching for these mutations in a population affected by environment will be a colossal work for the purpose of finding out risky individuals.

The likelihood of a mutation occurring and persisting in subsequent clones heavily depend on the efficiency with which toxic metabolites are produced and excreted from our body and also the efficiency with which small mistakes in DNA are rectified. Some polymorphic metabolic genes (i.e. low risk genes) play the role of "care takers" for these processes. Roles of these "care takers" genes vary strongly between individuals because of population variability in prevalence of polymorphic genes that regulate these processes. These polymorphic genes have subtle effect on cancer risk at individual level but may have a large population impact because

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the relevant polymorphism may be highly prevalent in a population. These "low-risk" genes can also become important determinants to assess population risk (Brennan 2002).

LOW-RISK GENES AND ITS IMPORTANCE

Phase I enzymes [e.g. genes of cytochrome P450 (CYP) family] are generally responsible for conversion of exogenous exposures into carcinogenic metabolites. Most of these metabolites are highly reactive with DNA and responsible for adduct formation and subsequent DNA mutation. Excretion of these intermediate metabolites requires action by phase II enzymes [e.g. glutathione S-transferase (GST) and N-acetyl transferase (NAT) families] to minimize the DNA-adduct formations (Bartsch et al. 2000; Strange and Fryer 1999). These phase I and II genes display polymorphisms and prevalence of the variant alleles are different in different population. Some of the variant alleles exhibit either low or high enzyme activities compared to the wild type alleles. Another potentially important source of inter-individual variability in relation to development of cancer is DNA repair capacity (e.g. XRCC1 gene). Polymorphisms in some cell cycle control genes (e.g. p53 codon 72, Cyclin D1) and repair genes, such as xPD, may also be involved in regulating apoptosis although the extent of functional differences in most cell cycle control and DNA repair polymorphisms is currently unclear (Seker et al. 2001). Similarly, genes involved in regulation and development of the immune system may be important for viral and haematopoietic neoplasm, although little data are available. Finally genes that influence behavior such as smoking, alcohol consumption and excess calorie intake have the potential to affect the cancer risk substantially. For example, several genes, including CYP2A6 and dopamine D2 receptor, have been suggested with the ability to quit smoking (Wu et al. 2000).

Functional variation in these genes, listed in Table 1, could predict the risk of cancer in a population in a better way than high-risk genes if the relevant polymorphism is highly prevalent in that population. For example, a polymorphism, which increases risk by 50% but also present in half of the population would account for 20% of all patients, similar to a high-risk gene with an increased risk of 5-fold, which is present in only 5% of the population (Table 2). Identification of

Table 1: A representative list of genes that may influence the risk of developing HNSCC

| Category of genes | Genes |
|--------------------------|--|
| Phase I metabolic genes | CYP1A1, CYP2A6, CYP2E1, CYP2D6, ADH2, ADH3 |
| Phase II metabolic genes | GSTM1, GSTT1, GSTP1, NAT1, NAT2, ALDH2 |
| DNA repair genes | XRCC1, XRCC3, xPD |
| Immune function genes | IL1A, IL2B, IL2, IL6 |
| Cell cycle control genes | p53, H-Ras |

(Brennan, 2002)

low-risk cancer genes may also be important for increasing our knowledge of carcinogenic process (Brennan 2002). Most environmental exposures (e.g. diet, tobacco, alcohol, air pollution) are complex mixtures and it is not clear yet how these exposures exert their effects. Identification of metabolizing and DNA repair genes involved in these exposures will help to clarify the process by which a cancer develops and may thus indirectly help to plan the prevention measure. For example, exposure to polycyclic aromatic hydrocarbons (PAHs) may be causes of HNSCC with possible exposure mainly from tobacco consumption. The glutathione S-transferase M1 and T1 genes code for enzymes which are involved in PAH metabolism and a role for these genes in HNSCC would enhance the credibility of a causal association with PAHs, specially if this association was restricted to smokers.

Table 2: Population attributable risk (PAR) and sample size requirements for various combinations of odd ratio (OR) and prevalence of polymorphism

| Prevalence % | OR | | | | |
|--------------|-------------|-------------|------------|-----------|-----------|
| | 1.25 | 1.5 | 2.0 | 5.0 | 25 |
| 50 | 11 | 20 | 33 | 66 | 92 |
| | <i>1267</i> | <i>387</i> | <i>136</i> | <i>30</i> | <i>13</i> |
| 20 | 4.8 | 9.1 | 16.7 | 44.0 | 82.8 |
| | <i>1850</i> | <i>535</i> | <i>172</i> | <i>28</i> | <i>8</i> |
| 5 | 1.2 | 2.4 | 4.8 | 16.6 | 54.5 |
| | <i>6020</i> | <i>1689</i> | <i>516</i> | <i>69</i> | <i>11</i> |

Population attributable risk (in bold) and sample size (in italic below PAR) required to detect each OR assuming a power of 80% and $p < 0.05$ (Brennan, 2002)

POLYMORPHISMS AND PREVALENCES IN POPULATIONS

GSTM1 and GSTT1

The Glutathione-s-transferases (GSTs) are

a family of enzymes known to play important roles in the detoxification of several carcinogens found in tobacco smoke. GSTs are dimeric proteins that catalyze conjugation reactions between glutathione and tobacco smoke substrates, such as aromatic heterocyclic radicals and epoxides. Conjugation facilitates excretion and thus constitutes a detoxification step. In addition to their role in phase II detoxification step, GSTs also modulate the induction of other enzymes and proteins involved in cellular functions, such as DNA repair (Hayes and Pulford 1995). This class of enzymes is therefore important for maintaining cellular genomic integrity and, as a result, may play an important role in cancer susceptibility.

GST enzymes are coded by at least five distinct loci, known as alpha, mu, pi, theta, and gamma. Two loci in particular, GSTM1 (mu-type) and GSTT1 (theta-type) may be of relevance for susceptibility to HNSCC. The GSTM1 and GSTT1 loci have been mapped on chromosomes 1p13.3 and 22q11.2, respectively. Deletion variants of GSTM1 and GSTT1 genes that result in loss of enzymatic activity, have been characterized. Three alleles have been identified at the GSTM1 locus: one is deletion allele and others are GSTM1a and GSTM1b that differ by C-G substitution. This substitution results in replacement of the amino acid, Lys, by the amino acid, Asn, at the position 172 in exon 7 of the protein. This Lys-Asn substitution results in no functional difference between the two alleles. As a result both GSTM1a and GSTM1b alleles are categorized as the positive conjugator phenotype. Two alleles have been identified at the GSTT1 locus – one wild type and the other one deletion-type (Pemble et al. 1994). Persons who are of the homozygous deletion genotype are categorized into the negative conjugator phenotype, while those who carry either one or both of the functional alleles are grouped into the positive conjugator phenotype (Rebbeck 1997; Hengstler et al. 1998).

These two genes have been studied extensively in different populations to find their association with the incidence of tobacco related cancer. An extensive review of gene variants for GSTM1 and GSTT1 has been published previously (Rebbeck 1997; Cotton et al. 2000) and will be updated briefly here. In the United States, the reported range of frequencies of GSTM1 homozygous deletion genotype varies

by ethnic group. Reported frequencies range 23 – 41% for individuals of African decent, 32 – 53% for individuals of Asian decent, 40 – 53% for those of Hispanic decent, and 35 – 62% for those of European decent (Rebbeck et al, 1999; Cotton et al, 2000). South American case-control studies have reported frequencies of 21% for Chileans (Quinones et al. 1999), 55% for Caucasian Brazilians, and 20% for Amazonian Brazilians (Arruda et al. 1998).

Groups such as Pacific Islanders and Malaysians have a reported GSTM1 homozygous deletion genotype frequency of 62 – 100%. Other Asian populations, such as the Japanese and Chinese, also have a high frequency of GSTM1 homozygous deletions and reported frequencies range 48 – 50% and 35 – 63%, respectively (Rebbeck 1997). Two Korean case-control studies reported frequencies of 53 and 56%, respectively, for the GSTM1 homozygous deletion genotype (Park et al. 2000; Kim et al. 2000). In India depending upon the ethnic population, GSTM1 homozygous deletion genotype varied from 20 to 79% (Roy et al. 2001)

Studies of GSTT1 null genotype demonstrate that, in the United States, deletion of GSTT1 is less common than the GSTM1 deletion genotype. Among those of European ancestry, 15-31% has GSTT1 homozygous null genotype. African-Americans have frequencies ranging from 22 to 29%, while those of Hispanic origin carry GSTT1 homozygous deletion in 10 – 12% of individuals (Rebbeck et al. 1999; Cotton et al. 2000; Gertig et al. 1998; Crump et al. 2000). One South American study reported that 19% of both Caucasian and Black Brazilians had the homozygous deletion genotype compared with 11% of Amazonian Brazilians (Arruda et al. 1998). Asians have the highest reported GSTT1 homozygous deletion genotype: 58% in Chinese, 38% in Malaysians and 42 – 46% in Korean populations, respectively (Lee et al. 1995; Park et al. 2000; Kim et al. 2000). In India we observed GSTT1 homozygous null genotype varied from 3-39% (Roy et al. 2001) in different populations.

CYP Genes

Human Cytochrome P450 that represent a large multigene family with differing substrate specificity is important in phase I activation reactions (El-Zein et al. 1997; Wu et al. 1997). They play important roles in the oxidative

metabolism of several pro-carcinogens to their ultimate reactive forms (Guengerich 1991).

CYP1A1: One member of the cytochrome P450 family, designated as CYP1A1 is of particular interest for its potential role in human lung and head and neck carcinogenesis (Nebert et al. 1991). The CYP1A1 gene that is located on chromosome 15q22-q24, encodes aryl hydrocarbon hydroxylase (AHH) involved in the activation of tobacco related procarcinogens, such as poly aromatic hydrocarbons (PAHs), nitrosamines and aromatic amines. CYP1A1 activity is also induced by PAH components of cigarette smoke condensate (McLemore et al. 1990; Anttila et al. 1991). There are four previously studied polymorphic sites in the human CYP1A1 gene. The CYP1A1 m1 polymorphism consists of a T-C substitution in the 3' noncoding region of the gene that creates a MspI restriction enzyme cleavage site. The CYP1A1 m2 polymorphisms is due to an A to G substitution at nucleotide 4889 in exon 7, that results in substitution of isoleucine by valine at the amino acid position 462 of the protein, a region that encodes a heme-binding domain of CYP1A1 (Hayashi et al. 1991). The CYP1A1 m3 polymorphism, creating a MspI site, is found only in African Americans (Crofts et al. 1993), so may not be important in association study. The CYP1A1 m4 polymorphism, C to A substitution, results in substitution of threonine by asparagine at 461 amino acid of the protein and is only two base pairs away from the m2 polymorphism and consequently has been less well studied phenotypically (Cascorbi et al. 1996).

Among control population, the frequency of the CYP1A1 MspI variant allele was reported as 37% in Japanese, 9% in Caucasians, and 42% in Hawaiians. We observed that 54% of individuals had at least one variant allele in Indian population (unpublished data). The reported CYP1A1 "Val" allele frequency has been observed 24% in Japanese, 3% in Caucasians, and 16% in Hawaiians (Le Marchand et al. 1998). In Indian population, 17-23% of the individuals had at least one variant allele at Ile/Val genotype (Sreelekha et al. 2001, our unpublished data).

CYP2E1: It activates carcinogenic N-nitrosamines, benzene, urethane, and other low molecular weight aromatic and heterocyclic compounds. This enzyme also metabolizes alcohol and is also inducible by ethanol (Oyama et al. 1997). The human CYP2E1 gene is func-

tionally well conserved but several polymorphic alleles have now been identified. The Restriction fragment length polymorphisms (RFLP) revealed that RsaI (G1259C) and PstI (C1091T) polymorphic sites are located in the 5' flanking transcription region of this gene and appear to be in complete linkage disequilibrium with each other (c1 wild-type allele and c2 rare/variant allele). Three genotypes of CYP2E1 resulted from digestion with restriction enzymes PstI and RsaI: a predominant wild-type homozygote c1/c1; the heterozygotes c1/c2 and rare homozygote c2/c2 are generally observed in different populations. Although the primary sequence of the enzyme is not altered, increased gene transcription has been suggested (Watanabe et al. 1994) by these polymorphisms. The substitution, T7668A, in intron 6 of the CYP2E1 gene has been characterized by a DraI RFLP (C rare/variant allele, D wild-type allele). The RsaI and DraI polymorphisms appear to be linked, i.e. individuals with the RsaI variant allele also had a variant DraI allele, although the reverse is not true (Hirvonen et al. 1993).

The distribution of c1/c1, c1/c2 and c2/c2 genotypes are 92%, 7% and 1% respectively among European-Americans and the C allele frequency is 10% in this population. South American population-based studies have reported frequencies of c1/c1, c1/c2 and c2/c2 genotypes as 71% and 28% and 1% among Mexican American, respectively. For African-American, c1/c1 and c1/c2 genotypes were 98% and 2%, respectively. The reported DraI variant allele frequencies among Mexican American and African American were 18% and 9% respectively (Omer et al. 2001). Asian case-control studies have indicated variation in the frequencies of c1/c1, c1/c2 genotypes and DraI variant allele of the CYP2E1 gene. Among the Taiwanese, the frequencies of c1/c1, c1/c2 and c2/c2 were 56%, 32% and 12%, respectively. Among the Japanese and Hawaiians, the frequencies of c1/c2 are 26% and 16%, respectively. The C allele frequencies are 25%, 33% and 15% for the Taiwanese, Japanese and Hawaiians respectively (Le Marchand et al. 1998). In Indian population, we observed the frequencies c1/c1, c1/c2 and variant genotype at DraI site as 98, 2 and 35%, respectively (unpublished data).

NAT Genes

Human are routinely exposed to highly

mutagenic and carcinogenic aromatic amines (including arylamines and heterocyclic amines) via smoking different types of tobacco, well-cooked food and other sources (Vineis 1994). Acetylation is an important route of biotransformation for these chemicals. In humans, two N-acetyltransferases (NATs), designated as NAT1 and NAT2, catalyze N- and O- acetylation of various arylamines. NAT2 and NAT1 are polymorphic enzymes that segregate independently into rapid and slow acetylator phenotypes (Weber and Hein 1985; Hughes et al. 1998). Genetic polymorphisms in the genes influence the metabolism of environmental arylamines and may modulate the risk of certain human cancers (Evans et al. 1983; Bell et al. 1995; Badawi et al. 1995, Taylor et al. 1998). An analysis of contribution of NAT polymorphisms to cancer susceptibility has been complicated by the presence of multiple alleles. Seven polymorphisms have been identified in the NAT1 gene, and more than 20 variant alleles of NAT2 have been described. The availability of good metabolic probes for NAT enzymatic activity has facilitated the assignment of a phenotype to the majority of the NAT2 variants.

In addition to the wild type (wt) NAT1*4 allele, seven variant NAT1 alleles (NAT1*3, *10, *11, *14, *15, *17, and *22) have been reported. The alleles NAT1 *14, *15, *17, *22 were considered to be phenotypically slow acetylating, whereas allele *10 was classified as a fast acetylating allele. NAT1 *4, *3 and 11 were classified as a normal acetylators. Classification of the subjects into fast (wt/fast or fast/fast), intermediate (wt/wt) or slow acetylators (slow/wt; no homozygous slow acetylators were found in the population) was derived from the genotype data according to current knowledge on the functional activity of the variant alleles. These were identified by conventional PCR RFLP based methods, whereas the polymorphic sites at nt 1088 and 1095 specific for NAT1* 10, *14 and *11 were identified using Light Cycler analysis. (Wikman et al. 2001). NAT2 alleles were determined using the PCR RFLP method using MspI, KpnI, BamHI, TaqI and DdeI. (Lin et al. 1993). This method determines the 5 most common wild type (NAT2*4) and slow activity (NAT2*5, *6, *7, *14) alleles. Individuals were considered phenotypically fast acetylators if they carry at least one NAT2* 4 allele.

In German population, the frequencies of

NAT1 fast, intermediate and slow acetylator genotypes were reported to be 3.7, 36.3 and 58.2%, respectively (Gonzales et al. 1998; Jourenkova-Mironova et al. 1999). In the same population, NAT2 slow, intermediate and fast acetylator genotypes were observed to be 56.8, 35.7 and 7.5%, respectively. Among Japanese, the frequency of NAT1 wild type allele *4 was reported 37.7% and the frequency of second most frequent allele, NAT1*10, was reported to be 21.3% (Katoh et al. 1999). The frequencies of NAT2 fast, intermediate and slow acetylators were 48.4, 45.9 and 5.7% respectively among Japanese population (Morita et al. 1999). The distributions of NAT2 fast, intermediate and slow acetylator among Chinese population are 35.5, 46.6, and 17.8%, respectively. So it could be noticed that NAT2 slow acetylators are highest frequent in Caucasians whereas the same are lowest frequent among Mongoloid population. So it is expected that pattern of association between NAT2 genotypes and risk of HNSCC might differ in Caucasian and Mongoloid populations.

Head and Neck Squamous Cell Carcinoma and Etiological Factors

HNSCC is a group of cancers defined by their location in oral cavity, pharynx, and larynx and common origin of squamous cell. For the year 1999, approximately 40,000 incident cases of HNSCC were diagnosed in the United States, and 20,400 deaths occurred from it (Landis et al. 1999). Worldwide, it has been estimated that approximately 500,000 incident cases are diagnosed each year (Parkin et al. 1999). Within the developing world, HNSCC represents the third most common among men and the fourth most common among women (La Vecchia et al. 1997).

Tobacco smoking is the strongest risk factors for HNSCC. Various population-based studies on male cigarette smokers have reported relative risks of 3-13 for ever smokers (Blot et al. 1996). When the amount of tobacco smoked is examined, a dose-response trend was demonstrated. Relative risks, adjusted for alcohol use, of 1.6 for light smokers (< 20 cigarettes per day for 20 or more years), and 4.4 for heavy smokers (>40 cigarettes per day for 20 or more years) have been found (Blot et al. 1996). In spite of a lower incidence compared with men,

it has been suggested that women have a relatively increased risk of HNSCC per tobacco smoke dose of carcinogens (Kabat et al. 1989; Muscat et al. 1996; Winn et al. 1981, 1982, 1986; Schlecht et al. 1999). Relative risks of 3.0 for light smokers, 4.4 for moderate smokers, and 10.2 for heavy smokers have been measured among women (Blot et al. 1996). Alcohol consumption is also linked to an increased risk of HNSCC. For those men and women who consumed more than 30 drinks of alcohol per week, the risk of developing HNSCC was nine times more than that of nondrinkers, odds ratios (OR) of 1.9, 2.3 and 9.1 have been demonstrated for light, moderate, and heavy alcohol consumers, respectively, compared with abstainers (Gillison et al. 2000).

Evidence of synergism was observed among persons who smoke tobacco and drink alcohol. Relative risks of approximately 40 have been found among those who smoke 40 or more cigarettes a day and consume 30 or more drinks per week (Blot et al. 1996). A recent case-control study conducted in Brazilian 784 cases of HNSCC measured a relative risk of 20 for those with the greatest cumulative measures of alcohol and tobacco (Gillison et al. 2000). It has been estimated that approximately 75 percent of the attributable risk of SCCHN result from the combined effects of tobacco and alcohol.

In India, oral cancer ranks first and third among all cancers in men and women respectively (Parkin et al. 1997). In south Asia, including India, oral cancer has been causally associated with smoking and chewing of tobacco alone or together with betel quid (BQ), whereas in Western countries cigarette smoking and heavy alcohol consumption are the main etiological factors (La Vecchia et al. 1997). Unlike tobacco smoke, chewing of tobacco with BQ results in exposure, to a major extent, to carcinogenic tobacco-specific nitrosamines (TSNA) and, to a minor extent, to nitrosamines derived from areca nut alkaloids. It is also reported that chewing of BQ also generates high amount of reactive oxygen species (ROS) in mouth. It has been implicated in the initiation, promotion and progression of carcinogenesis. Thus TSNA and ROS are the major genotoxic agents involved in chewing related oral cancer (Nair et al. 1999).

Variant Genotypes and HNSCC

Most tobacco carcinogens are metabolized via complex enzymatic mechanisms involving both activation and detoxification reactions. Individual's susceptibility to cancer may be partly explained by variability in enzymatic activities of phase I and phase II metabolic genes. Therefore variations in the expression of these genes due to heritable genetic polymorphisms, might modulate the process of carcinogenesis by altering the exposure levels of tobacco derived carcinogens. Here we have summarized results of some recent studies on different ethnic populations, to show how the variant forms of different genes are associated with risk of HNSCC among tobacco users.

GSTM1

The earliest published study of GSTM1 and HNSCC of which we are aware focused on the enzyme phenotype. A study of laryngeal cancer reported an OR of 2.5 (95 percent CI: 1.8-3.1) for persons lacking phenotypic expression of the GSTM1 enzyme (LaFuente et al. 1993). Results published later by the same group reported an OR of 1.9 (95 percent CI: 1.2-3.1) for GSTM1 null phenotype and risk of larynx cancer (LaFuente et al. 1998). Coutelle et al. (1997) reported an OR of 4.7 (95 percent CI: 1.0-21.8) for larynx cancer among those who lacked GSTM1 enzyme expression and an OR of 1.8 (95 percent CI: 0.5-6.2) for cancer of the oral cavity or pharynx after adjustment for age.

Many studies examined the risk of HNSCC and the GSTM1 deletion genotype with reported ORs between 0.9 and 3.9 for the GSTM1 deletion polymorphism in world-wide different populations (Deakin et al. 1996; Hung et al. 1997; Park et al. 1997; Gonzalez et al. 1998; Matthias et al. 1998; Ophuis et al. 1998; Worrall et al. 1998; Jourenkova-Mironova et al. 1999; Morita et al. 1999; Tanimoto et al. 1999; Olshan et al. 2000; McWilliams et al. 2000; Hamel et al. 2000; Jahnke et al. 1996; Trizna et al. 1995; Kihara et al. 1997; Jourenkova et al. 1998; Cheng et al. 1999; Katoh et al. 1999; Sato et al. 1999; Park et al. 2000). In Indian populations, studies also reported that GSTM1 homozygous null genotype enhances the risk of oral cancer among tobacco smokers and chewers (Buch et al. 2002; Sikdar et al. 2003).

But another report could not observe enhancement of oral cancer risk due to presence of GSTM1 homozygous null genotypes in tobacco users (Sreelekha et al. 2001)

GSTT1

Many studies have examined the GSTT1 deletion genotype and risk of HNSCC. Only a few studies suggested an increase in risk, with ORs ranging from 1.4 to 2.6 (Matthias et al. 1998; Jourenkova-Mironova et al. 1999; Hamel et al. 2000; Trizna et al. 1995; Jourenkova et al. 1998; Cheng et al. 1999). Other studies, however, have reported ORs of 0.5 to 1.2 (Deakin et al. 1996; Hung et al. 1997; Ophuis et al. 1998; Worrall et al. 1998; Olshan et al. 2000; McWilliams et al. 2000; Jahnke et al. 1996; Katoh et al. 1999). In Indian populations, one study reported positive association between GSTT1 null homozygous genotype and risk of oral cancer among tobacco users (Buch et al. 2002) while the others did not observe this association (Sreelekha et al. 2001; Sikdar et al. 2003).

CYP1A1

Molecular epidemiological studies of CYP1A1 variants have linked the m1 and m2 polymorphisms to smoking related cancers of the lung, head and neck, and esophagus in different populations (Bartsch et al. 2000). There was over representation of the CYP1A1 "Val" variant allele among Caucasian patients with oral cancer. Similarly, an increased prevalence of the CYP1A1 Val-Val variant genotype was found among Japanese patients with head and neck cancer (Morita et al. 1999). Individuals with the homozygous CYP1A1 MspI variant genotype were at significantly increased risk for oral squamous cell carcinoma, in particular after exposure to low concentrations of PAH. The combination of homozygous CYP1A1 MspI variant and GSTM1 homozygous null genotype increased the risk. The buccal mucosa and upper gingiva appear to be the most susceptibility tissues in carriers of the risk genotype (Tanimoto et al. 1999). One study on Indian population reported high risk of oral cancer among tobacco users with CYP1A1 "Val" allele at the polymorphic site (Sreelekha et al. 2001) while our study could observe this association with another CYP1A1 allele (Sikdar et al. 2003).

CYP2E1

Only a few studies, done so far, showed association between head and neck cancer and CYP2E1 variant genotypes in different populations (Hung et al. 1997; Bouchardy et al. 2000; Tan et al. 2000). Our study on a small sample size could not show positive association between risk of oral cancer and polymorphism in this gene (Sikdar et al. 2003).

NAT Genes

Examination of NAT1 and NAT2 polymorphisms in Japanese patients with oral squamous cell carcinoma suggest that the NAT1 *10 variant allele increases an individual's risk for this disease (Katoh et al. 1998). Another Japanese study described that the NAT2 slow-acetylator genotype predisposed individuals to higher risk of HNSCC among tobacco smokers (Morita et al. 1999), supporting similar observations among Caucasians (Gonzales et al. 1998; Jourenkova-Mironova et al. 1999).

FUTURE PERSPECTIVES

The literature on association between genetic polymorphism and cancer risk is vast but the knowledge obtained from this effort is however disappointing. Many studies reported both positive and negative associations between variant alleles and risk of cancer in the same ethnic population. This lack of consistency is probably due to small sample size and mixed ethnicity in the samples. Previous investigations were based on a relatively small sample size (nearly, case-150 and control-150) involving one or few genes. Future studies should include much larger samples of case and controls (e.g. more than 1000 samples) and simultaneous analysis of large numbers of candidate genes. Interaction between susceptibility genes and endogenous (e.g. hormones) and exogenous (e.g. tobacco smoke, betel quid etc.) risk factors should be studied in greater details knowing the biological functions of the variant alleles. Most of the conclusions on genetic susceptibility and HNSCC were drawn from case-control studies only. Family-based design has been proposed for genotyping of not only the affected individuals but also their parents and siblings as well. These family based un-affected

individuals will provide ethnically matched controls and also may nullify other confounding factors.

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