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Polymorphism at CYP and GST Gene Loci and Susceptibility to Tobacco Related Cancers

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Exposure to synthetic or natural chemical compounds present in the environment is causally associated with a large proportion of human cancers. Xenobiotic metabolizing enzymes (XMEs) are responsible for the metabolism of many of these exogenous chemicals that are toxic, mutagenic and/ or carcinogenic. The XMEs comprise of phase I enzymes that are involved in the bioactivation of several carcinogens, and phase II enzymes that take part in detoxification by conjugating the electrophilic compounds formed by the phase I enzymes with small bio-molecules (e.g. glutathione). Hence, the toxicological outcome of exposure, absorption and activation/detoxification of xenobiotes depends on a delicate balance between the phase I and phase II enzymes. Polymorphism at genes encoding these enzymes can thus result in altered metabolism and result in toxicity or mutagenicity thereby altering individual susceptibility to diseases caused by environmental agents. Many studies have been conducted on the potential association between polymorphic expression of *CYP1A1, CYP2D6, GSTM1, GSTM3, GSTP1, GSTT1* and various types of environmentally induced cancers, particularly those that are related to tobacco abuse. *CYP1A1, CYP2D6* and *CYP2E1* polymorphic alleles have been associated with susceptibility to lung, and head and neck cancers. Apart from modulating head and neck cancer risk, polymorphisms at *GSTM1, GSTM3, GSTP1* and *GSTT1* gene loci have also been involved in a variety of tobacco - associated cancers like lung, bladder and oesophageal cancer. The relationship between polymorphisms at metabolic gene loci and cancer risk is known to vary in distinct ethnic groups. This suggests that the variant alleles can not serve as universal biomarkers of susceptibility to environmental carcinogens. Studies on polymorphism at CYP and GST gene loci that have identified host factors responsible for modifying susceptibility to tobacco-related cancers are documented in this review.

Key Words: Polymorphism in *CYP1A1, CYP2D6, CYP2E1, GSTM1, GSTM3, GSTP1, GSTT1* genes, Tobacco-related cancers

Introduction

Epidemiological studies have shown that up to 90% of all cancers are related to environmental factors, with tobacco smoke and diet being the main attributable exposures (IARC 1990). Tobacco use is causally associated with non-communicable diseases, including cancers of the lung, bladder, head and neck and oesophagus. Globally, almost half a million head and neck cancers are diagnosed every year, with three fourths of these occurring in the developing world (Parkin et al. 1993). Oral cancer ranks as the 6th most common cancer in the world and has a particularly high incidence in South East Asian countries (Johnson 1991, Parkin et al. 1997). Globally, oral cancer is the third most common cancer in males, while in females it ranks fourth (Parkin et al. 1993). In India, it is in fact the leading cancer site among males as reported by various cancer registries in the country (ICMR 1992).

Cigarette smoking is causally associated with high incidence of lung cancer in western countries. The two most important etiological factors, implicated in the development of oral cancer in western populations, are tobacco smoking and alcohol consumption. Both, tobacco smoking and tobacco chewing, with or without betel quid have

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been implicated in the high prevalence of oral cancer in the Indian population (IARC 1985, 1986). For the Indian population, the proportion of oral cancer cases attributable to tobacco use – both smoking and chewing - has been estimated at 81% in males and 36% in females, the range being 61% - 70% (Notani et al. 2000). Although there is overwhelming evidence of the etiological association between tobacco and oral cancer, the majority of the tobacco habitues do not suffer from cancer (Ng et al. 1993). This is because genetic host factors can interact with environmental carcinogens to place an individual at a greater or lesser risk of a particular cancer than another individual (Pelkonen 1992, Raunio & Pelkonen 1994). The most likely candidates that could influence individual cancer susceptibility are genes encoding xenobiotic- metabolizing enzymes (XMEs).

Xenobiotic Metabolizing Enzymes

The majority of chemical carcinogens require metabolic activation before they can interact with cellular macromolecules and cause cancer initiation. The xenobiotic- metabolizing machinery consists of two types of enzymes, with the phase I enzyme reactions resulting in the creation of functional groups and reactive centers on substrates (e.g. -OH, $-NH_2$, -SH, -COOH) and phase II conjugation enzymes inactivating these substrates. The principal enzymes responsible for phase I reactions belong to the cytochrome P-450 multigene family (*CYPs*), although other enzyme systems such as the flavin - containing monooxygenase, alcohol dehydrogenases and prostaglandin synthetase may also serve this function for certain substrates. The phase I reactions involve the introduction of an oxygen atom into toxic, lipophilic chemicals as the first step toward detoxification and elimination (Guengerich & Shimada 1991). The resulting increase in hydrophilicity facilitates further metabolic processing and excretion. As an unfortunate consequence of these reactions, certain chemicals are not detoxified but are activated to their ultimate carcinogenic form. This pathway is the primary step in the activation of the majority of chemical carcinogens. The hydrophilicity of the intermediate metabolite formed by the phase I enzyme is further increased by phase II reactions. During phase II reactions, membrane - bound and cytosolic enzymes including glutathione S-transferases (*GSTs*), UDP-glucuronosyl transferases, acetyl transferases and sulfotransferases attach highly water soluble moieties to the polar groups introduced by phase I reactions. Thus, the coordinated expression and regulation of phase I and phase II drug - metabolizing enzymes and their metabolic balance are important host factors in determining the outcome of exposure to carcinogens.

Cytochrome P-450 Monooxygenases (*CYPs*):

The *CYPs* are divided into 14 gene families of which *CYP1*, *CYP2* and *CYP3* are primarily active in the metabolism of a wide range of chemicals including drugs, environmental chemicals and pollutants. *CYPs* are predominantly expressed in the liver, although specific isozymes are also expressed in many extra-hepatic tissues, including the lung, kidney and gastro-intestinal tract (Raunio et al. 1995). The expression of specific *CYP* isozymes is often restricted to a particular cell type, and can therefore result in tissue and cell - specific toxicity as a consequence of *CYP* - mediated activation of toxic or mutagenic substrates. The *CYPs* that are known to exhibit polymorphism are *CYP1A1*, *CYP2A6*, *CYP2C9*, *CYP2C19*, *CYP2D6* and *CYP2E1*. Of these, polymorphisms at the *CYP1A1*, *CYP2D6* and *CYP2E1* genes have been studied in relation to susceptibility to tobacco-related head and neck cancers.

CYP1A1

CYP1A1 is located on human chromosome 15q22-q24, and encodes aromatic hydrocarbon hydroxylase (AHH). This enzyme catalyzes the first oxidative step in the metabolism of PAHs [such as benzo(a)pyrene] to carcinogens (Shimada et al. 1989) and is expressed predominantly in extra-hepatic tissues including lung (Anttila et al. 1992). Individual variation in *CYP1A1* inducibility, determined by measuring AHH activity, is well documented. At present, four different sequence polymorphisms have been identified in the *CYP1A1* gene. The first allelic variant known as *CYP1A1**2 involves a $T_{6235} \rightarrow C$ transition in the 3′ non-coding region of *CYP1A1*, leading to the introduction of an *Msp*I restriction site (wild type: m1; mutant allele:

m2) (Kawajiri et al. 1990). The *CYP1A1*3* variant results from an A_{4889}→G transition in exon 7 resulting in an Ile_{462}→Val substitution in the heme-binding region of *CYP1A1* (Hayashi et al. 1991). The presence of this polymorphism confers a 3-fold increase in the catalytic activity of the AHH enzyme in Asians (Cosma et al. 1993, Crofts et al. 1994). The African specific polymorphism, *CYP1A1*4*, located in intron 7, involves a transition of T_{5639}→C resulting in an *Msp*I restriction site (Crofts et al. 1993). Another polymorphism designated *CYP1A1*5* has recently been detected in exon 7 and results in a Thr_{461}→Asn substitution due to a C_{4887}→A mutation (Cascorbi et al. 1996). The phenotypic consequences of the *CYP1A1*4* and *CYP1A1*5* polymorphisms are as yet unknown.

*CYP1A1*2* and *CYP1A1*3* have been studied extensively in relation to lung cancer susceptibility. Strong correlation has been reported between lung cancer risk and homozygosity for the *CYP1A1* variant alleles in the Japanese (Kawajiri et al. 1990, Kawajiri et al. 1993, Nakachi et al. 1993, Sugimura et al. 1998) but not among Caucasians cigarette smokers (Tefre et al. 1991, Hirvonen et al. 1992, Alexandrie et al. 1994, Bouchardy et al. 1997). An association with increased risk for oesophageal cancer has also been reported (Nimura et al. 1997).

There have been relatively very few studies on the relationship between *CYP1A1* variant alleles and susceptibility to head and neck cancers caused by chronic tobacco smoking or chewing. The results obtained by Morita et al. (1999) demonstrated that the Val/Val genotype enhances risk for head and neck SCC (HNSCC), especially for pharyngeal cancer (OR: 4.1, 95% CI: 1.1-15.0 and OR: 5.7, 95% CI: 1.1-28.0, respectively). Only 4 studies in the Japanese population have focused on oral cancer. Katoh et al. (1999) have reported that the *CYP1A1* Val allele has no effect on risk of cancer of the lip and oral cavity in the Japanese. However, Sato et al. (999) and Tanimoto et al. (1999) have demonstrated that the m2 homozygous variant genotype is related to increased risk for oral squamous cell carcinoma (SCC) especially at low smoking doses (OR: 4.3, 95% CI: 1.9-10.1 and OR: 7.0, 95% CI: 1.8-27.4, respectively).

Three studies on the *CYP1A1* variant alleles and susceptibility to HNSCC have been reported among Caucasians. The first of these studies, conducted by Park et al. (1997) concluded that the *CYP1A1* Val allele was significantly higher in cases as compared to controls (OR: 2.6, 95% CI: 1.2-5.7). Another study by Oude et al. (1998) indicated that neither the *CYP1A1* m2 nor the Val allele was linked to increased susceptibility to HNSCC. The latest report by Olshan et al. (2000) also failed to find any association between the *CYP1A1*3* polymorphism and susceptibility to HNSCCs including cancers of the oral cavity, pharynx and larynx. No association was found between the *CYP1A1*3* allele and oral cancer risk among Indian tobacco chewers and bidi or cigarette smokers in a study carried out by us. Interestingly, the *CYP1A1*2* mutant allele was found to elevate oral cancer risk in individuals habituated to both bidi/cigarette smoking and tobacco chewing (Bhisey et al., unpublished data).

CYP2D6

The second *CYP* gene extensively studied in relation to tobacco-related cancers is *CYP2D6*. This gene is located on human chromosome 22q13.1 and encodes an enzyme popularly known as debrisoquine hydroxylase. It is expressed mainly in the liver although its presence has also been demonstrated in the brain and intestine (Raunio et al. 1995). It is responsible for the metabolism of over 40 drugs including anti-arrythmics (e.g. propafenone, flecainamide), anti-depressants (e.g. desipramine, amitryptiline) and substances of abuse such as MDMA (ecstacy) (Smith et al. 1995 and references therein). NNK, a tobacco-specific nitrosamine, has also been reported to be a substrate for *CYP2D6* (Crespi et al. 1991). *CYP2D6* has been found to be genetically polymorphic (Mahgoub et al. 1977) and several allelic variants of the *CYP2D6* gene have been described (Daly et al. 1996). Individuals inheriting two copies of the mutant alleles cannot produce the enzyme and are unable to metabolize *CYP2D6* substrates and are therefore termed as poor metabolizers (PM). Certain individuals have the entire *CYP2D6* gene deleted (*CYP2D6*5*) while some inherit multiple copies of a *CYP2D6* variant sequence (Johansson et al. 1993). The most commonly studied *CYP2D6* alleles are *CYP2D6*3* and ***CYP2D6*4***. The *CYP2D6*3* allele consists of a single base pair deletion in the coding

sequence in exon 5 causing a frameshift. *CYP2D6*4* also results in a frameshift but this mutation is characterized by a base substitution in the splice site at the intron 3/ exon 4 boundary that results in the loss of the first base in exon 4. *CYP2D6*4* is observed in 80% of all PMs among Caucasians while *CYP2D6*5* and *CYP2D6*3* form 10% and 5% of the PMs (Wolf et al. 1992).

Phenotyping studies have reported that the *CYP2D6* extensive metabolizer phenotype is linked with high lung and bladder cancer incidence (Ayesh et al. 1984, Kaisary et al. 1987, Caporaso et al. 1989, 1990, Gough et al. 1990, Idle et al. 1992, Roots et al. 1992). On the other hand, the *CYP2D6* PM genotype was found to be associated with increased risk for cancer at other sites (Ladero et al. 1991, Elexpuru-Camiruaga et al. 1995). A report by Wolf et al. (1992), that investigated several different cancers, showed that the *CYP2D6*4* mutant allele was weakly associated with increased overall cancer risk. Thus, on the whole, studies on the potential association between polymorphic expression of *CYP2D6* and incidence of various types of cancers have yielded conflicting results (Smith et al. 1995,Nebert et al. 1996).

The possibility of *CYP2D6* being related to individual susceptibility to oral cancer has been explored in very few studies. A German study on HNSCCs, including oral, pharyngeal and laryngeal SCCs (Matthias et al. 1998) showed that no cancer risk was associated with polymorphism at the *CYP2D6* gene locus. In a study among tobacco habitués too, the *CYP2D6*4* polymorphism did not influence oral cancer risk in any of the habit group. A Croatian study has also excluded a role for *CYP2D6* polymorphism in head and neck cancers. However, an investigation from the UK (Worrall et al. 1998) has shown that the frequency of the homozygous mutant alleles was significantly higher in oral cancer patients as compared to controls (OR: 3.2; 95% CI: 1.6-6.5). Thus, *CYP2D6* could be involved in the pathway for detoxification of tobacco carcinogens. The difference in risk reported by various groups could be due to differences in the nature of carcinogen exposure caused by variation in the composition of the tobacco used for smoking or chewing.

CYP2E1

CYP2E1 is the third *CYP* gene of interest with respect to cancers caused by tobacco abuse. It is located on chromosome 10q24.3-q-ter (Koble 1993) and is expressed mainly in the liver although its presence has also been detected in the lung, vascular endothelium and brain (Raunio et al. 1995). The ethanol inducible *CYP2E1* enzyme metabolizes several known tobacco-smoke carcinogens including tobacco-specific nitrosamines like NNK, NNN and NNAL, benzene, styrenes, butadiene and urethane (Raunio et al. 1995b). Several polymorphisms have been identified in the human *CYP2E1* gene. Two RFLPs with restriction enzymes *Pst*I and *Rsa*I lie in the 5′ promoter region of the gene. These polymorphisms are in complete linkage disequilibrium with each other and result in increased transcription of the gene (Watanabe et al. 1994). Other variant alleles reported are a $T_{7668} \rightarrow A$ substitution in the 6th intron of the gene resulting in the formation of a *Dra*I restriction site, a *Taq*I RFLP due to a $C_{9930} \rightarrow G$ transversion, and a *Rsa*I RFLP as a consequence of a mutation in intron 5 of *CYP2E1* (McBride et al. 1987, Uematsu et al. 1991). No phenotypic change has been associated with the 3 mutations. Two other polymorphisms have been described in the coding region of the gene. The first leads to a $His_{76} \rightarrow Arg$ substitution while the second substitutes isoleucine for valine in exon 8. The phenotypic consequences of these alleles and association with disease susceptibility are currently under study.

Several contradictory results have been reported with respect to both *CYP2E1 Dra*I and *Pst*I/*Rsa*I polymorphisms (Bartsch et al. 2000 and references therein). The *Dra*I wild genotype was found to be associated with an increased risk for lung cancer in different ethnic groups (Uematsu et al. 1994, Le Marchand et al. 1998, Wu et al. 1998). On the other hand, a large number of studies have revealed a lack of association between the *Dra*I polymorphism and bladder cancer, while two studies in Caucasian women showed that the *CYP2E1 Dra*I variant, conferred a higher risk for renal carcinoma and breast cancer in premenopausal smokers (Sheilds et al. 1996, Farker et al. 1998). The *CYP2E1 Pst*I/*Rsa*I mutant allele (c2) has been associated with decreased risk of lung cancer

(Wu et al. 1997, Le Marchand et al. 1998). However, a Chinese study has shown that the c2 allele is associated with increased risk of oesophageal cancer (Lin et al. 1998) among smokers.

Data from five studies indicated that *CYP2E1* polymorphism was not associated with head and neck cancer risk. Lucas et al. (1996) have shown that there is no association between either the *Dra*I or *Rsa*I polymorphism and risk of upper aerodigestive tract cancers among French Caucasians. Two other groups (Gonzalez et al. 1998, Matthais et al. 1998) also failed to detect any association between risk for head and neck cancers (including cancer of the oral cavity) and the *CYP2E1 Pst*I/*Rsa*I or *Dra*I polymorphisms among Caucasians. A Japanese study on oral cancer at several different sites also showed similar results for the *Pst*I/*Rsa*I polymorphisms (Morita et al. 1999). However, a Chinese study has reported that the *Rsa*I variant allele is a strong risk factor for oral cancer (OR: 4.7, 95% CI: 1.1-20.2) among individuals who smoked but did not chew betel quid (Hung et al. 1997).

Glutathione S-Transferases (*GSTs*)

Among phase II XMEs, the *GST* class of enzymes have received a great deal of attention owing to their importance in detoxification of tobacco carcinogens such as PAH diol epoxides, aromatic amines, hydrazines and products of oxidative stress. *GSTs* are classified into five different families - four are cytosolic while one is microsomal. Cytosolic *GSTs* are a superfamily of enzymes involved in the conjugation of a wide range of electrophilic substrates with the abundant cellular nucleophile-glutathione (GSH), thereby facilitating their metabolism, detoxification and excretion (Chasseaud 1979). These dimeric proteins are further divided into four classes - α, μ, π and θ. Various workers have investigated the role of *GST* polymorphisms in modulating individual susceptibility to head and neck cancers. Polymorphic variants have been observed in four *GST* genes, namely, *GSTM1*, *GSTM3*, *GSTP1* and *GSTT1*. Three of these, *GSTM1*, *GSTM3* and *GSTP1* are involved in the detoxification of PAH diol epoxides while *GSTT1* participates in the detoxification of potentially carcinogenic monohalomethanes and reactive epoxide metabolites of butadiene, both of which are constituents of tobacco smoke. Hence, several workers have investigated polymorphism at these gene loci with respect to individual susceptibility to head and neck cancers.

GSTM1

The *GSTM1* gene belongs to the μ class of enzymes and is located on chromosome 1p13.3. The substrates preferred by *GSTM1* are benzo(a)pyrene-4,5-oxide, (+)-anti-benzo(a)pyrene-7,8-diol-9,10-oxide, aflatoxin B1-8,9-epoxide, styrene oxide, *trans* stilbene oxide, and oxygen free radicals. This gene is reported to be deleted in ~50% of Caucasians resulting in the absence of the enzyme (Seidegard et al. 1988). In addition to the null genotype, two functional alleles denoted as *GSTM1*A* and *GSTM1*B* have been described. The *GSTM1* A and B alleles differ by a $C_{534} \rightarrow G$ transversion, resulting in a $Lys_{172} \rightarrow Asn$ substitution (Seidegard et al. 1990). The functional differences between these two alleles have not yet been elucidated.

Several studies have investigated the relationship between the *GSTM1* null genotype and lung cancer risk. A recent meta-analysis on 12 case control studies reported a small increase in lung cancer risk (OR: 1.4, 95% CI: 1.2–1.6) associated with the *GSTM1* null genotype (McWilliams 1995). However, when the studies were stratified by race, an elevated OR was detected in the Japanese population (OR: 1.6, 95% CI: 1.3-2.1) but not in Caucasians (OR: 1.2, 95% CI: 0.9-1.4). In fact many studies on the Japanese have shown a 3 to 7-fold increase in lung cancer risk in *GSTM1* null individuals (Rebbeck 1997 and references therein). Furthermore, Strange et al. (1991) have reported that white Americans without a functional *GSTM1* enzyme have a three-fold higher risk for adenocarcinoma of the stomach and colon as compared to those with the *GSTM1* positive genotype. The *GSTM1* null genotype has also been associated with increased bladder cancer risk (Daly et al. 1993, Lafuente et al. 1993, Katoh et al. 1995).

Polymorphism at the *GSTM1* gene locus has been studied extensively in relation to head and neck cancers, including cancers of larynx, pharynx and oesophagus. Most of these studies have

indicated that the *GSTM1* null genotype does not modulate individual risk for these cancers (Katoh 1994, Deakin et al. 1996, Hung et al. 1997, Jahnke et al. 1996, Park et al. 1997, Jourenkova et al. 1998, Oude-Ophuis et al. 1998, Olshan et al. 2000, To-Figueras et al. 2000). However, Oude Ophius and co-workers (1998) have shown that the *CYP1A1* m2 and *GSTM1* null combined genotype was significantly over-represented among patients with benign head and neck lesions while individuals having a combination of the *GSTM1* null and *CYP1A1* Ile/Val genotype showed an excess risk for the development of HNSCC (OR: 2.6, 95% CI: 0.7-10.3) (Olshan et al. 2000). On the other hand, three studies have reported the *GSTM1* null genotype as a susceptibility factor in head and neck cancers (Trizna et al. 1995, Kihara et al. 1997, Nimura et al. 1997).

Conflicting reports exist on the association between the *GSTM1* null genotype and susceptibility to oral cancer among Japanese and Caucasians. No association was observed between the *GSTM1* null genotype and oral cancer risk in 8 of 13 studies, 2 of which were in the Japanese population (Katoh 1994, 1999), while 6 involved white Americans and Europeans (Park et al. 1997, Worrall et al. 1998, Matthias et al. 1998b, 1999, Jahnke et al. 1999, Jourenkova et al. 2000, Olshan et al. 2000). However, a positive association between the *GSTM1* null genotype and oral cancer risk was observed in 4 Japanese studies (Katoh 1999, 2000, Sato et al. 1999, 2000) and in 1 Caucasian study (Park et al. 2000). Katoh and his group have reported an OR of 1.8 (95% CI 1.0 – 3.3) after adjusting for age and cigarette smoking habit (Katoh 2000) while Sato et al. (2000) reported an OR of 2.24 (95% CI 1.4 – 3.6). An earlier study by Sato's group had also reported a statistically significant increase in the frequency of the combined *CYP1A1* m2/m2 and *GSTM1* null genotype among patients with oral SCC as compared to controls (Sato et al. 1999). In a study of African-American patients with cancer of the oral cavity, Park et al. (2000) reported an OR of 3.1 (95% CI 1.1 – 8.5). Besides, heavy cigarette smokers with the *GSTM1* null genotype were reported to be at an increased risk for laryngeal and oral cancer (Jourenkova et al. 1998, 1999, Cheng et al. 1999, Olshan et al. 2000). These studies reported crude ORs in the range of 2.0 – 3.0. Investigations among an Indian population revealed that GSTM1 null genotype is a major risk factor for development of cancer of the buccal mucosa. Increased life time exposure to chewing tobacco was associated with a further increase in oral cancer risk in GSTM1 null individuals (Buch et al 2002). Thus, in different studies, the *GSTM1* null genotype was found to confer weak to moderate oral cancer risk in distinct ethnic groups.

GSTM3

Another polymorphic member of the *GST* μ family, *GSTM3*, is also located on chromosome 1p (Inskip et al. 1995, Patskovsky et al. 1999). *GSTM3* is the most abundant *GST* expressed in the human lung (Anttila et al. 1993) and metabolizes substrates like hydrogen peroxide and cumene hydroperoxide. The wild type *GSTM3* allele is termed *GSTM3*A*. The variant allele, termed *GSTM3*B*, carries a 3 bp deletion in intron 6 that results in the generation of a recognition sequence for the YY1 transcription factor (Inskip et al. 1995). Both negative and positive regulatory functions have been ascribed to the *GSTM3* B allele although the functional consequence of the two alleles has not been investigated (Inskip et al. 1995, Yengi et al. 1996).

People with low expression of *GSTM3* were previously observed to be at an increased risk of developing adenocarcinoma of the lung (Anttila et al. 1995). Genotyping studies had indicated that individuals who are homozygous or heterozygous for the *GSTM3*B* allele have a higher risk for cancers of the larynx (Jahnke et al. 1996) and lung (Matthias et al. 1998), than do individuals with the homozygous wild type *GSTM3*A* genotype. A subsequent study with a larger sample size reported that *GSTM3* B allele is a protective factor for larynx cancer (Jahnke et al. 1999). This premise has been validated by three recent studies (Jourenkova et al. 1999, Matthias et al. 1998, 1999). However, the *GSTM3* A or B allele was not implicated as a risk factor for oral cancer in all the reports so far available (Worrall et al. 1998, Jourenkova et al. 1999, Matthias et al. 1999 Buch et al. 2002). Recently, the *GSTM3* AA and the *GSTM1* null genotype combination has been implicated in susceptibility to head and neck cancers (Jourenkova et al. 1999) and lung cancer (To- Figueras et al. 2000).

GSTT1

GSTT1, present on chromosome 22q11.2, encodes a member of the *GST* θ class of enzymes. The substrates of *GSTT1* include 1,2,3,4-diepoxybutane, 1,3-dichloroacetone, epibromohydrin, 1,2-dibromoethane and 1,2-epoxy-3-(4′-nitrophenoxy) propane. The inability of 40% Caucasians to conjugate methyl chloride led to the conjecture that a polymorphism was present in the theta class of *GST* enzymes (Peter et al. 1989). The *GSTT1* gene polymorphism was later shown to be the result of a 480 bp deletion, leading to the absence of the *GSTT1* enzyme activity (Pemble et al. 1994).

The association between the *GSTT1* null genotype and oral cancer risk is not unequivocal. Two Japanese studies (Katoh 1999, Sato et al. 1999) and 8 reports among Caucasians (Park et al. 1997, Worrall et al. 1998, Matthias et al. 1998, 1999, Olshan et al. 2000, Jourenkova et al. 1998, Trizna et al. 1995, Deakin et al. 1996) did not observe any association between the *GSTT1* null genotype and oral cancer. However, 2 studies associated the *GSTT1* null genotype with increased risk for cancer of the oral cavity and HNSCC (Jourenkova et al. 1999, Cheng et al. 1999). Jourenkova and co-workers (1999) have reported that the risk conferred by the GSTT1 null genotype for oropharyngeal cancer (OR: 2.0, 95% CI: 1.0-4.0) was further elevated in individuals with a long (>30 years) smoking history (OR: 3.3, 95% CI: 1.3-8.1). Buch et al (2002) also did not find a significant difference in the distribution of GSTT1 null genotypes among oral cancer patients and controls. The GSTT1 gene deletion was also found to confer a 2.27-fold risk (95% CI: 1.43-3.60) for HNSCC (Cheng et al. 1999). The same group observed a further increase in risk in individuals null for both GSTT1 and GSTM1 (OR: 3.64, 95% CI: 1.94-6.84). The *GSTT1* null genotype has been implicated in susceptibility to colorectal cancer Deakin et al. 1996), while *GSTT1* null individuals carrying the *CYP1A1* m2 allele were found to incur higher risk for renal call carcinoma (Longuemaux et al. 1999). In another study, the GSTT1 wild genotype was found to be associated with increased risk for bladder cancer among non-smokers (Brockmoller et al. 1996).

GSTP1

The fourth polymorphic *GST* gene – *GSTP1* - is located on chromosome 11q13 and is widely expressed in normal human epithelial tissues. In addition to the *GSTP1*A* wild- type allele, *GSTP1* has two variant alleles, *GSTP1*B* and *GSTP1*C*. *GSTP1*B* has an A_{313}→G transition in exon 5 causing Ile_{104}→Val amino acid change. In addition to this base substitution, *GSTP1*C* has a C_{341}→T transition resulting in an Ala_{113}→Val substitution. These two polymorphisms result in decreased enzyme activity (Ali-Osman et al. 1997).

The *GSTP1* polymorphism has been studied in a variety of cancers. The frequency of the *GSTP1*B* wild type allele was significantly higher in oesophageal cancer patients (Morita et al. 1998) An association between the *GSTP1* mutant allele and elevated risk for lung and bladder cancer has also been observed (Harries et al. 1997, Ryberg et al. 1997). The cancer group that has been most widely studied with respect to the *GSTP1* polymorphism is HNSCC. Most of the studies have linked the *GSTP1* variant allele to increased oral cancer risk. Park and co-workers(1999) have found that individuals homozygous for the *GSTP1* polymorphic alleles were at an increased risk for oral cancer (OR: 2.4, 95% CI: 1.2-4.8), and the risk was still higher in those reporting low smoking exposure (OR: 3.4, 95% CI: 1.1-11). A similar risk has been noted in both Caucasians (OR: 2.6, 95% CI: 1.1-6.2) and African-Americans (OR: 2.3, 95% CI: 0.68-7.5). Matthias et al. (1999b) have shown significant differences between pharyngeal cancer patients and controls with respect to the *GSTP1* polymorphism, while Olshan et al. (2000) report only a marginal increase in risk (OR: 1.2, 95% CI: 0.8-1.9). A moderate risk of 2.78 (95% CI: 1.06-7.51) associated with this polymorphism was noted in non-smoking Japanese oral cancer patients (Katoh et al. 1999b) while Caucasian oropharyngeal cancer patients with > 30 years smoking history were reported to have a 2-fold risk (95% CI 1.0-3.9) (Jourenkova-Mironova et al. 1999). On the other hand, Morita et al (1999) and Jahnke et al. (1999) found that *GSTP1* wild genotype is more frequent in laryngeal and oropharyngeal carcinoma cases, respectively.

Future Prospects

Characterization of genetic determinants of cancer susceptibility is important in the understanding of disease pathogenesis and to design individualized cancer prevention strategies. Growing evidence indicates the existence of several groups of predisposing polymorphic genes, such as those involved in carcinogen metabolism and DNA repair, that can lead to increased cancer risk particularly among people exposed to environmental genotoxicants and carcinogens present in tobacco products. In designing preventive strategies, it is necessary to identify vulnerable members, particularly those faced with the unfortunate combination of exposure to carcinogens and a cancer - predisposing genotype. The profile of polymorphism at cancer - predisposing genes can be used in clinics to identify individuals with greater susceptibility to cancer. Genetic risk factors could also be evaluated for their involvement in the progression of premalignant conditions like oral leukoplakia and submucous fibrosis to oral cancer. Another clinically important avenue that can be explored is the identification of biomarkers that could help pinpoint patients at a higher risk for the development of a secondary lesion. More importantly, it would help in developing better preventive strategies and individualizing cancer therapies that would, hopefully, lead to alleviation of a great deal of human suffering.

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