Discussion

You do not really understand something unless you can explain it to your grandmother.

Albert Einstein
Malignancy of UADT remains a significant health issue especially in a developing country like India. Despite the advances in diagnosis and treatment modalities for malignancies of UADT including chemotherapy, radiotherapy, surgery and various prevention strategies, it has resulted in little or no improvements in the morbidity or mortality rates due to poor survival rates. Furthermore, UADT cancers show heterogeneity in their clinical behavior which cannot be predicted using the current clinical markers such as primary tumor size and status of lymph nodes, alone. In this thesis, we investigated the role of genetic basis of complex traits such as susceptibility and outcome associated with the initiation and progression of UADT cancers that might help in providing valid information to the existing knowledge in this field. This is the first report on the association between CYP1A1*2A, CYP1A1*2C, GSTM1, GSTT1, GSTP1-313A/G, CYP2E1*5B and CYP2E1*6 gene polymorphisms and risk of UADT cancers in the Tamilian population of south India. It is also one of the largest Indian case-control studies on UADT cancers; while the association of CYP2E1*1B and ABCB1 3435C>T gene polymorphisms and UADT cancer risk is the first report from the Asian population.

6.1. Genetic polymorphisms of drug metabolizing enzymes, CYP1A1, CYP2E1, GST and transporter protein, ABCB1 on the risk of UADT cancers

As the CYP1A1 is involved in the metabolic activation of various tobacco related procarcinogens to potential carcinogens, it is reasonable to speculate that a carcinogen activating enzyme with increased activity could be associated with an elevated risk of developing cancer. Thus, we have selected CYP1A1, an important phase I enzyme to investigate the relationship between their polymorphisms and development of UADT cancers in an ethnic Indian population.

In our study, significant association between UADT cancer risk and CYP1A1*2A variant genotypes was observed. Our results suggest a linear increase in risk for the
heterozygous and homozygous variant genotypes that could reflect intermediate and higher levels of enzyme activity. The CYP1A1 *1A/*2A and *2A/*2A contributed to nearly 2-fold and 4-fold overall increased risk respectively, towards the development of UADT cancers. However, a significant association was not found for the CYP1A1*2C genotypes and risk of UADT cancers. There have been various molecular epidemiological studies describing inconsistent reports on the CYP1A1 polymorphisms and risk of UADT cancers. In a Japanese study, patients carrying CYP1A1*2C Val/Val genotype had an increased risk for pharyngeal cancer while high prevalence of CYP1A1*2A heterozygous and homozygous variant genotypes were reported among oral squamous cell carcinoma patients in another study conducted in Japan. Also, the risk associated with mutant allele of CYP1A1*2A was found to vary among the different subsites of oral cavity. The two other studies on Japanese oral squamous cell carcinoma have reported significant associations for CYP1A1 *2A/*2A and CYP1A1 Val/Val genotypes. The oral cancer risk was enhanced among Koreans carrying CYP1A1*2A homozygous mutant genotype. A study conducted in Germans did not find an association of CYP1A1 presumed risk genotypes and UADT cancers. In another German study, no association was found between the CYP1A1*2C variant genotypes and the risk of oral cavity cancer. A significant association between CYP1A1 Val allele and increased risk for oral cancer risk was observed in an American based study, while another study did not find an association between CYP1A1 Ile/Val and head and neck cancer risk. A Brazilian study reported that the CYP1A1*2C variant genotypes did not have a significant role in the oral cancer risk while another study in the Brazilian population found a 2.4-fold increased risk for head and neck cancers associated with polymorphic CYP1A1*2A and GSTM1 null genotypes, eventhough a significant risk was not observed with CYP1A1*2A genotype alone.

A study conducted in Keralite population (98 cases and 60 controls) of south India reported high risk of oral cancer among carriers of CYP1A1*2C polymorphism. In
another study done in Kolkata population of eastern India (80 cases and 67 controls), did not find any association for CYP1A1*2A polymorphism and oral squamous cell carcinoma. Both the reports are in contrast to our findings. However, the present study with a larger sample size compared to other Indian studies suggests that CYP1A1*2A, but not CYP1A1*2C polymorphism may be significantly associated with the overall risk of UADT cancers in Tamilians. The reason for the contradictions between our results and those by other Indian studies could be due to ethnic differences in cultural, linguistic and dietary practices in the Indian population or it might be due to the increased sample size of the present study.

When the analyses were done based on the site of carcinoma, a significant association between all the three anatomical carcinoma sites and CYP1A1*2A variant genotypes were observed. A 3-fold increased risk was noted among oral carcinoma subjects while the risk was more than 5-fold among pharyngeal carcinoma patients carrying CYP1A1*2A/*2A genotypes. For the cancers in the laryngeal region, the associated risk among the carriers of *1A/*2A and *2A/*2A was more than 3 and 6-fold respectively. This difference in the risk among the anatomical carcinoma sites might be due to the differential exposure of upper aerodigestive tract towards variety of environmental pollutants as smoking plays a substantial role in intrinsic laryngeal cancer while the alcohol consumption is more significant in the aetiology of oral cavity, pharyngeal and extrinsic laryngeal tumours. Furthermore, the extent and size of the transitional cells of larynx varies with level of tobacco consumption and in extreme cases, it results in squamous metaplasia throughout the laryngeal region.

Our results suggest that the genetic polymorphism in CYP1A1*2A is a strong predisposing risk factor for UADT cancers in the Tamilian population of south India. The results also indicate that the risk varies between the cancer subsites of upper aerodigestive tract for carriers of CYP1A1*2A mutant genotypes. Nevertheless, the
CYP1A1*2C polymorphism is less likely to be associated with UADT cancers in the population studied.

The CYP2E1 enzyme investigated in the study is of particular interest as this is involved in the metabolic activation of procarcinogens into reactive intermediates capable of forming adducts and damaging DNA and play an essential role in chemical carcinogenesis. In the study, the frequency distribution of CYP2E1 genotypes was not different among the case and control groups, suggesting that the polymorphisms of CYP2E1 independently do not play a role in the susceptibility to the cancer development. A significant risk was not observed for CYP2E1 genotypes based on the site of carcinoma as well. Our results were in agreement with an Indian study done in Kolkata population which did not find a positive association between Pst I site of CYP2E1 and oral squamous cell carcinoma risk. A German based Caucasian study also supports our finding which did not observe an association between upper aerodigestive tract cancers and CYP2E1*5B and CYP2E1*6 risk genotypes. Our results suggest that polymorphisms in CYP2E1 alone may not play a significant role in the development of UADT cancers in the study population.

GSTs that belong to superfamily of phase II enzymes are involved in the conjugation of a wide range of electrophilic substances thereby facilitating detoxification. Among the isoenzymes analyzed, GSTT1 was found to play a significant role in the etiology of UADT carcinogenesis in the population studied. The deficient GSTT1 genotype carriers had nearly a 3-fold increased risk for UADT cancers, while GSTM1 null and variant genotypes of GSTP1 were not significantly associated with the cancer risk. Absence of GSTT1 isoenzyme in patients with GSTT1 null genotypes might have resulted in failure of the detoxification of carcinogens and environmental toxins, thereby increasing the risk for UADT cancers. The role of GST genotypes in the subsites of UADT cancers showed that there was a significant increased risk of 4.5 and nearly 3.5-fold for laryngeal and
oral cavity cancers respectively among carriers of GSTT1 null genotype. The risk was reduced to 2.4-fold in pharyngeal cancers compared to oral cavity and laryngeal cancers.

GSTs are mainly involved in the detoxification of a wide variety of potentially toxic and carcinogenic electrophiles by conjugating with glutathione. They are also involved in the deactivation of oxidative metabolites of endogenous or exogenous carcinogenic agents (industrial chemicals, dietary compounds, tobacco products, drugs and environmental carcinogens) that are probably associated with UADT cancer risk. Individuals with altered form of the enzymes (null genotypes of GSTM1 or GSTT1 and the Ile/Val or Val/Val genotypes of GSTP1) cannot detoxify the activated carcinogen leading to the progression of cancer. Induction of other enzymes and proteins important for cellular function, e.g. DNA repair is also modulated by GSTs. Hence, they are important in cancer susceptibility and also for maintaining cellular genomic integrity. The study in Tamilian population of south India had shown that the deletion of GSTT1 genotype is a strong predisposing risk factor for UADT cancers.

Apart from xenobiotic metabolizing enzymes, polymorphisms in the genes encoding for xenobiotics and drug transporters may also contribute to the risk and clinical outcome of UADT cancers. The analysis of xenobiotic transporter protein, ABCB1 3435C>T in UADT carcinoma cases had revealed that the homozygous mutant genotype, TT of ABCB1 was significantly associated in developing the cancers as evidenced by more than a 2-fold overall increased risk. The increased C allele frequency in controls conferred protection from carcinogens and toxins while the increased T allele frequency in cases might have led to decreased P-gp activity resulting in increased susceptibility to UADT cancers. The findings were compatible with the previous studies that investigated ABCB1 3435C>T polymorphism and disease susceptibility and have concluded that the polymorphism may contribute to the risk for renal epithelial tumor.
colon carcinoma\textsuperscript{250} and ulcerative colitis\textsuperscript{251}. Nevertheless, further analysis with other polymorphisms of \textit{ABCB1} gene, especially non-synonymous G2677T/A polymorphism which is partially linked with 3435C>T polymorphism may be needed to finally assess the impact of \textit{ABCB1} polymorphisms in the study population.

The influence of \textit{ABCB1} based upon the individual carcinoma sites revealed a definite role of the gene in causing pharyngeal cancers with nearly a 4-fold increased risk. A significant risk was not observed among laryngeal cancers, while the oral cavity cancers approached significance (P=0.08). The P-gp expression in salivary glands is well documented\textsuperscript{278}. But, there is no significant evidence to state that P-gp is expressed in oral cavity, pharynx and larynx. Nonetheless, our results suggest that the \textit{ABCB1} 3435C>T polymorphic TT genotype might have a distinct role in the etiology of oropharyngeal cancers.

The controls and patients were in Hardy-Weinberg equilibrium for all the genotypes analyzed, indicating that the population had undergone random mating.

\textbf{6.2. Gene-gene interactions of \textit{CYP1A1}, \textit{CYP2E1}, \textit{GST} and \textit{ABCB1} associated with UADT cancers}

In complex polygenic diseases such as UADT cancers, it is likely that genetic susceptibility is dependent on the action of several gene polymorphisms operating in concert. Polymorphisms in individual gene may impart only to a small extent, and it is likely that the cumulative effect of many polymorphisms will be more important in its pathogenesis. Studies showing associations between genotypes and susceptibility are largely based on the effects of single genes. They consider therefore, main rather than interactive effects of a gene even though the main effect could be undetectable at times while an interactive effect could be large.
The gene-gene interactions apparent with the combinations of multilocus deficient GST genotypes modulated the risk associated with UADT cancer susceptibility than the risk related to a single GST deletion genotype. The risk associated with GSTT1 null genotype in the presence as well as in the absence of GSTM1 genotype depicts the independent involvement of GSTT1 gene deletion in the etiology of UADT cancers. The observation was consistent with a previously reported meta-analysis evaluating nine head and neck cancer studies for the combined effects of deficient GSTM1 and GSTT1 genotypes. The decreased risk noticed when GSTP1 wild genotype combined with GSTT1 null genotype might be due to the fact that tobacco or alcohol derived carcinogens and toxins are multiple substrates for GSTP1. This was confirmed by the increased risk observed among carriers of GSTP1 polymorphic variants along with GSTT1 null genotype. In addition, a significant association was observed for concurrent deletion of the GSTM1, GSTT1 genes and mutant genotypes of GSTP1 indicating that individuals having a defective genotype for more than one of these genes would therefore be at greater risk.

Several carcinogens, including PAHs present in the tobacco smoke are reported to be detoxified by GSTs. Similar results were reported for various combinations of GST polymorphisms and susceptibility to oral leukoplakia, larynx cancers and head and neck cancers. An Indian study had reported 2-fold risk associated with head and neck cancer patients carrying the GSTM1 and GSTT1 null genotypes individually, though the risk was not significant with multivariate logistic regression model analysis. The wild type genotype of GSTP1 in combination with GSTM1 null or GSTT1 null genotype increased the susceptibility to 2.5 and 2.8-folds respectively for the cancers. The risk was further increased to nearly 4.5-fold, when the combinations of GSTM1 null, GSTT1 null and wild type genotype of GSTP1 was analysed. Identification of individuals with null genotypes of GSTs may eventually assist in the prevention of UADT cancers by allowing early detection of individuals with a high risk. Therefore, the
deficiency of GST genes especially in combinations is important in public health issues related to UADT cancers.

The interaction between phase II deficient enzymes and a phase I hyperactive enzyme (CYP1A1) is of interest as it may result in a larger amount of toxic compounds that might have a role in the initiation or progression of UADT cancers. In the present study, the wild type genotypes of CYP1A1*2A and GSTM1 taken together as baseline, the risk associated with the combinations of the mutant genotypes of CYP1A1*2A and deficient GSTM1 were determined. Individuals with combined mutant genotypes of CYP1A1*2A and GSTM1 null genotype had an increased risk for cancers that was higher than the risk elevation observed for the susceptible genotypes of CYP1A1 or GSTM1 gene alone. The interaction between CYP1A1 and GSTM1 is of interest as GSTM1 null may be associated with CYP1A1 inducibility. Thus, our findings were in accordance with Japanese and Korean studies that indicated the definite role of CYP1A1*2A and GSTM1 deficient genotypes in the oral carcinogenesis. Similarly, the combined effects of mutant variants of CYP1A1*2A and deletion genotype of GSTT1 resulted in high risk for the carcinogenesis. A lung cancer study done in a south Indian population had reported high risk associated with combinations of variant or null genotypes of CYP1A1*2A, GSTM1 and GSTT1 that further supports our findings. While the individuals deficient for both GST genes in presence of mutant genotypes of CYP1A1*2A were at remarkably high risk than individuals who had either of the deletion along with CYP1A1*2A mutant genotypes.

These observations indicate that metabolic imbalance of activation and detoxification of CYP1A1*2A and GST deletion genotypes respectively worked synergistically for UADT cancer risk. The initial step in the metabolic activation of PAHs is mediated by cytochrome P450 gene products, mainly the isozyme, CYP1A1. In addition to the metabolic activation of PAHs by CYP1A1 enzyme, it is also involved in the bioactivation
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of several other tobacco related procarcinogens such as nitrosamines and aromatic amines to carcinogenic products\textsuperscript{155,166}. The carcinogenic metabolites produced by the hyperactive CYP1A1 enzyme failed to be eliminated as a result of the lack of GST genes that are mainly involved in the detoxification of carcinogenic substances. Interindividual differences in the rate of metabolism (activation/detoxification) have been thought to be due to genetic polymorphisms.

Among the CYP2E1 genotypes, polymorphic genotypes of CYP2E1*1B have observed to produce significant interactions among the GST deficient individuals. Interestingly, eventhough, GSTM1 or CYP2E1*1B genotypes when analyzed alone did not show a significant association with UADT cancers, the combined analysis of these genotypes showed a significant interaction corresponding to nearly a 4-fold increased risk. Similarly, CYP2E1*1B along with GSTT1, further increased the risk to more than 4.5-fold while both the GST deletion genotypes and CYP2E1*1B had a remarkably high interaction that contributed to nearly 7-fold risk in the initiation of UADT cancers. The interactions noticed along with the deficient GST might be due to the enhanced activity of CYP2E1 enzyme associated with polymorphism in CYP2E1*1B\textsuperscript{207}.

There are no studies reporting on the interaction of CYP2E1*1B and other presumed genotypes which indeed to be confirmed in other ethnic groups would be of clinical significance. However, in the present study, no significant interactions were observed for CYP2E1*5B and CYP2E1*6 when combined with GST genotypes. A Japanese study reported an interaction between CYP2E1*5B and GSTM1 null genotype and gastric cancer\textsuperscript{283}. On the contrary, a Chinese study reported that the GSTM1 non-null genotype had a synergistic effect along with CYP2E1 c1/c1 genotype causing an 8.5-fold risk to esophageal cancer\textsuperscript{284}. However, another Chinese study could not find a positive interaction between CYP2E1*5B and CYP2E1*6 in the presence of different isoenzymes of GST on the risk to precancerous gastric lesions\textsuperscript{285}. 

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Eventhough an interaction was not observed when either of CYP1A1*2A or CYP1A1*2C genotypes were combined with variant GSTP1, a significant interaction with triple genotype combinations of CYP1A1 (*2A & *2C) and variant alleles of GSTP1 contributed to nearly a 3-fold risk in the development of UADT cancers. The interaction explains the increased enzyme activity associated with the CYP enzymes and the reduced enzyme activity that affected the detoxification of electrophilic metabolites that resulted in DNA-adduct formation and carcinogenesis. Cancer is a complex disease in which multiple loci are involved and only the combined analysis of several markers could provide new clues for prediction of its occurrence. These positive findings suggest that interindividual variation in the metabolic pathways involved in the functionalization and detoxification of specific xenobiotics is an important susceptibility factor for UADT cancers in Tamilian population.

The main activity that occurs during the phase III elimination is the excretion of the parent drugs, metabolites, and xenobiotics from the cell via the transmembrane efflux pump, P-gp\textsuperscript{17}. In the present study, the subjects carrying the transporter protein, ABCB1 C3435C>T and deficient GSTT1 had more than 6-fold increased risk which was higher than the risk observed for individual polymorphic genes. Furthermore, these mutant genotypes along with variant GSTP1 had a 20-fold risk in our population. On the other hand, the combined analysis of the genes associated with metabolic activation and elimination pathways have shown an enhanced risk for polymorphic ABCB1, GSTM1 and CYP1A1*2A genes. A remarkable 11-fold higher risk was observed, when deficient GSTT1 was replaced with GSTM1 in the presence of ABCB1 and CYP1A1*2A polymorphic genes. The variant forms of the three genes (ABCB1, GSTT1 and CYP1A1*2A) have been shown to play a significant role in the etiology of UADT cancers when it was separately analyzed. Therefore, the individuals carrying these polymorphic
genotypes that are mainly involved in metabolic activation as well as elimination are highly susceptible to develop cancers associated with UADT. In addition, the interaction observed for polymorphic ABCB1, GSTT1 and CYP1A1*2C having a 7-fold risk also shows the role of metabolic activation of various procarcinogens by mutant forms of CYP1A1*2C genotypes. Therefore, our study indicates that individuals polymorphic for CYP1A1*2A, GSTT1 and ABCB1 genes have a genetically high risk for UADT cancers, and combined genotypes of these susceptible genes revealed higher risk than that ascribed to a single susceptible gene.

The enhanced enzymatic activity associated with CYP2E1*1B in the presence of ABCB1 & GSTM1 and ABCB1 & GSTT1 could be related to the significant role of ethanol inducible CYP2E1 and its involvement in the metabolism of tobacco specific nitrosamines to its ultimate DNA binding carcinogenic forms. The interactions observed for the polymorphic genotypes of CYP2E1*1B along with ABCB1 and GSTT1 was higher than the interactions observed when the GSTT1 was replaced with GSTM1. Therefore, the findings clearly illustrate that the CYP2E1*1B mutant genotypes along with polymorphic genotypes of the elimination pathways have a definite role in the pathogenesis of UADT cancers. It also explains the risk related in the presence of a single malfunctioning elimination system compared to the increased risk associated with multiple detoxification or transport pathways that are polymorphic in the accumulation of carcinogens in the body which would result in the transformation of normal cells to neoplastic cells.

The CYP2E1*6 genotypes did not show a significant role in the UADT carcinogenesis when it was analyzed individually as well as with other genotypes of CYP2E1 or different isoenzymes of GST. Interestingly, among the individuals who were carriers of polymorphic CYP2E1*6, ABCB1 and GSTT1 had a significant role in the development of UADT cancers which was evident by a risk of more than 7-fold. Hence, our findings
indicate that the risk associated with the multiple elimination pathways of xenobiotics in the presence of polymorphic genes involved in the metabolic activation of procarcinogens do have a definite role in the UADT carcinogenesis. The structure activity analyses of various transport substrates of P-gp have revealed that, it is associated with the elimination of hydroxylated carcinogenic metabolites. However, we did not find any published work that demonstrated an interaction of phase I and II along with phase III presumed genotypes especially ABCB1 3435C>T which need to be confirmed in other ethnic groups. By evaluating the associations between genotypes and disease phenotype, it may reflect a link between at-risk genotypes, alone or in combination, to identify subjects who are poor metabolizers and consequently more likely to suffer formation of carcinogen-DNA adducts and initiation of carcinogenesis.

6.3. Gene-environment interactions associated with UADT cancers

The environmental factors like smoking, alcohol, tobacco chewing and occupational exposure to toxins and carcinogens are responsible for almost 90% of all the cancers. Cancers of the UADT are relatively common forms of malignancy in the low socioeconomic group with high risk factors, such as tobacco smoking, tobacco chewing and alcohol abuse, poor oral hygiene and nutritional deficiency. In India, tobacco and alcohol have been proven as the major risk factors in the etiology of UADT cancers. The involvement of these factors along with the genetic polymorphism has a major role in the etiology of the UADT cancers. For any given environmental exposure, individual differences in the susceptibility might have a genetic basis. Therefore, the genetic variability enzymes involved in metabolic activation, detoxification of environmental carcinogens or the polymorphism in the xenobiotic transporter protein may partially explain host susceptibility to these cancers.
Although the individuals homozygous for CYP1A1*2A mutant genotype had an increased susceptibility to UADT cancers, a significant interaction was observed with increased exposure among regular chewers carrying CYP1A1*2A/*2A genotype. However, the result obtained may be interpreted in the presence of the fact that the number of controls was less compared to the cases when the subgroup analysis was carried out. The results of our studies did not suggest a significant interaction between tobacco smoking or alcohol consumption and CYP1A1 genotypes on the multiplicative scale.

An earlier study from a south Indian population had reported a high risk of oral cancer among the tobacco users carrying CYP1A1*2C polymorphism\textsuperscript{187}. Another Indian study, reported a significant risk for oral cancer among tobacco users carrying both CYP1A1*2A homozygous variant and GSTM1 null genotypes, even though a significant association was not found with CYP1A1*2A genotypes alone\textsuperscript{186}. In a Japanese study, CYP1A1*2A homozygous variant genotype was significantly associated with increased risk of oral squamous cell carcinoma at low levels of cigarette dose. They found that among the patient group, the mean smoking index for the individuals homozygous for the rare genotype was significantly less than the smoking index for patients with the wild type genotype\textsuperscript{179}. Two other separate studies among Japanese have shown that the susceptibility to oral squamous cell carcinoma was remarkably higher at a lower smoking dose level among those carrying rare mutant genotypes of both CYP1A1*2A and CYP1A*2C genotypes\textsuperscript{181,182}. However, American based studies did not find significant interactions between CYP1A1*2C polymorphism and tobacco use either on the multiplicative or additive scales, even though the polymorphic carriers were at an increased risk for oral cancer in a study\textsuperscript{176,177}. A Caucasian study also did not find a significant interaction between CYP1A1*2A and CYP1A*2C polymorphic genotypes and smoking and alcohol consumption on the risk to upper aerodigestive tract cancers\textsuperscript{174}. A study from Italy did not find CYP1A1*2A polymorphism as a risk factor in the head and
neck cancers and also any associated gene-environment interactions related to alcohol consumption or tobacco smoking\textsuperscript{105}.

In India and other Asian countries, oral cancer has also been associated with chewing of tobacco with BQ. The oral use of smokeless tobacco is widely prevalent in India; the different methods of consumption include chewing, sucking and applying tobacco preparations to the teeth and gums. The chewers used tobacco in the form of betel quid that consists of betel leaf (\textit{Piper betle} L), betel or areca nut (\textit{Areca catechu} L), slaked lime [Ca (OH)\textsubscript{2}], catechu (\textit{Acacia catechu} L) and tobacco. The chewing of tobacco with BQ results in exposure to carcinogenic TSNA\textsubscript{s} and nitrosamines derived from areca nut alkaloids. It has also been reported that the chewing of BQ generates high amount of ROS in mouth that is implicated in multistage carcinogenesis. Thus TSNA\textsubscript{s} and ROS are the major genotoxic agents involved in chewing related UADT cancers\textsuperscript{109,111}.

The difference in the risk among the anatomical carcinoma sites noticed in the study might be due to the differential exposure of upper aerodigestive tract towards variety of environmental pollutants especially among the tobacco chewers as evidenced by the significant interaction among the individuals polymorphic for CYP1A1*2A genotypes. Therefore, the findings indicate that the genetic polymorphism in CYP1A1*2A is not only a strong independent predisposing risk factor for UADT cancers, but also an enhanced risk factor among the regular tobacco chewers carrying *2A/*2A genotypes.

In the present study, even though we did not find an overall risk for CYP2E1*1B, CYP2E1*5B, CYP2E1*6 genotypes and UADT cancers, significant gene-environment interactions were observed which might contribute to the cancer risk when individuals carrying mutant genotypes were exposed to high risk environmental factors. We noticed a modifying effect of alcohol consumption on CYP2E1*1B, which is a potent inducer of CYP2E1 gene. Alcohol enhances the penetration of carcinogenic compounds by
facilitating the uptake of environmental carcinogens especially from tobacco smoke through the mucosal membranes that are damaged by the direct effect of alcohol\textsuperscript{126}. The enhanced penetration of carcinogens might be the reason for the interaction observed among regular alcoholics carrying CYP2E1*1B mutant genotypes.

Similarly, significant interactions were also noted among smokers and tobacco chewers carrying mutant genotypes of CYP2E1*1B and CYP2E1*6. The world's highest incidence of tobacco related cancers were reported among central, south, and northeast India\textsuperscript{54}. In our study population, tobacco was smoked as cigarettes or in the form of bidi especially among the rural population. However, bidi was found to be the most prevalent form of smoking in India compared to cigarettes. The involvement of CYP2E1 in the metabolic activation of tobacco specific nitrosamines could be the reason for the interaction observed in the present study. According to the epidemiological studies, 90% of cancers are associated with impact of acquired environmental factors. Both the inherited and acquired susceptibility play a vital role in cancer initiation, which can be associated with changes in CYP2E1 enzyme activity\textsuperscript{288}.

There are various molecular epidemiological studies describing inconsistent reports on CYP2E1 polymorphisms and risk of UADT cancers associated with gene-environment interactions. An Indian study done in Kolkata population could not find a positive association between Pst I site of CYP2E1 and oral squamous cell carcinoma risk among the tobacco users (80 oral cancer patients and 67 controls)\textsuperscript{188}. The findings of our study were in agreement with a Japanese study which found a significant interaction among smokers carrying CYP2E1*6 polymorphic genotype in causing oral squamous cell carcinoma. However, an interaction was not observed with alcohol consumption and the polymorphic genotype\textsuperscript{215}. A Brazilian study found an increased risk of 3.6-fold for oral cancer associated with CYP2E1 Pst I polymorphism\textsuperscript{184}. A Chinese study showed a significantly increased oral cancer risk for CYP2E1 c1/c2 and c2/c2 genotypes.
compared with the c1/c1 genotype among those who did not chew betel quid (OR 4.7; 95% CI 1.1-20.2)\textsuperscript{214}. Significant differences in the distribution of genotypes or haplotypes among Italian head and neck cancer patients carrying homozygous variant forms of CYP2E1*5B and CYP2E1*6 genotypes as well as gene-environment interactions were not observed\textsuperscript{185}. In a Caucasian study, patients carrying rare alleles of CYP2E1*5B and CYP2E1*6 had 7 and 6 fold increased risk for oral cavity/pharyngeal cancers among the heaviest drinkers (>80 g/day) compared with lighter drinkers with other genotypes\textsuperscript{213}. However, another German based Caucasian study, did not observe a significant interaction between CYP2E1*5B and CYP2E1*6 at-risk genotypes and smoking and alcohol consumption in the susceptibility to upper aerodigestive tract cancers\textsuperscript{474}. In another Kolkata based Indian study, C allele of CYP2E1*6 enhanced the susceptibility to leukoplakia among the tobacco users. But, a positive association was not found among CYP2E1*5B genotype carriers\textsuperscript{189}. Our results suggest that, polymorphisms in CYP2E1 may not play an independant role in the development of UADT cancers. Nevertheless, the significant gene-environment interactions observed for polymorphic CYP2E1*1B and CYP2E1*6 genotypes, might contribute to the risk for developing UADT cancers in the study population.

The significant gene-environment interactions that further modify the susceptibility to UADT cancers were noted. Significant interactions were observed among the tobacco chewers who were polymorphic for GST genes under study. Our findings did not suggest a strong interaction between smoking, GSTT1 and GSTM1 null genotypes. Three different studies\textsuperscript{186,188,231} conducted in eastern (Kolkata) and western (Mumbai) parts of India, reported that GSTM1 null genotype is a risk factor for the development of oral cancer among tobacco users. One of the above studies also reported that deletion of GSTT1 emerged as a protective factor against the risk of oral cancer especially among the tobacco chewers which was contradictory to our finding\textsuperscript{186}. However, another Indian study showed that those carrying deficient GSTT1 gene were at a higher risk for
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oral cancer and the risk was further increased in smokers carrying the deletion genotype\textsuperscript{232}. Another Indian study observed significant gene-environment interactions with \textit{GSTM1} null and \textit{GSTT1} null genotypes in tobacco chewers (3.5 and 2.2-folds respectively). A 4-fold increase in the risk was reported in the patients who were habitual alcoholics carrying deficient \textit{GSTM1} genotype\textsuperscript{233}. Another Indian study done in Keralites\textsuperscript{187} (a south Indian population) had showed that neither \textit{GSTM1} null nor \textit{GSTT1} null significantly contributed to the susceptibility to oral cavity carcinoma among tobacco users. However in the present study, a significant interaction was noticed only among smokers (>40 PY) carrying \textit{GSTP1} polymorphism. The \textit{GSTP1} enzyme that is involved in the detoxification of benzo (a) pyrene diol epoxide and acrolein present in cigarette smoke\textsuperscript{28} might have enhanced the UADT cancer risk among smokers (>40PY) carrying \textit{GSTP1} polymorphic genotypes. A German study reported that polymorphism at \textit{GSTP1} mediates susceptibility to squamous cell carcinoma of the upper aerodigestive tract as they found to have a decreased frequency of Ile/Ile genotype in cancer patients compared to controls\textsuperscript{28}. Eventhough the polymorphic \textit{GSTP1} genotypes did not show an overall risk to UADT cancers, the significant gene-environment interactions in the presence of potential hazardous environmental factors suggest the essential role of the polymorphic gene on the UADT carcinogenesis in the study population. The chronic use of alcohol consumption has been implicated as a risk factor for cancers of UADT where it acts as a solvent and enhances the penetration of carcinogens into the mucosa\textsuperscript{120,289}. The \textit{GSTT1} deletion among the regular alcoholics might have exacerbated the UADT cancer pathogenesis compared to other GST genes.

In the Brazilian population, a 2-fold risk was associated with \textit{GSTM1} null genotype in the development of head and neck cancers among tobacco users and alcoholics. The oral cavity and pharyngeal cancers have an increased risk of 3 and 2-folds respectively in the population\textsuperscript{184}. A study conducted in Italy did not find \textit{GSTM1} and \textit{GSTT1} deletion genotypes as significant risk factors in the susceptibility to head and neck cancers. The
study had also reported the absence of the gene-environment interactions associated with the cancer in the population\textsuperscript{195}. In a study done in Chinese population, smokers, alcoholics and tobacco chewers having null genotypes of \textit{GSTM1} and/or \textit{GSTT1} had a significantly increased oral cancer risk compared with those who had non-null genotypes of both \textit{GSTM1} and \textit{GSTT1}\textsuperscript{214}. A study conducted in US smokers have shown that \textit{GSTM1} and \textit{GSTT1} null genotypes are independent risk factors for squamous cell carcinoma of head and neck and depicted as markers for genetic susceptibility to tobacco-induced tumorigenesis\textsuperscript{220}.

Our results partially support these findings as we could observe only a few interactions associated with \textit{GSTM1} and \textit{GSTT1} genes which may be explained on the basis of inter ethnic differences. A German study found that \textit{GSTM1} null did not have a significant role in contributing to the risk of oral cancer\textsuperscript{175}. Another German study reported the negative role of \textit{GSTT1} null in UADT cancer risk and they also found that the \textit{GSTM1} A/B genotype confers a protective role in the cancer susceptibility\textsuperscript{174}. An American study reported a negative association between \textit{GSTM1} null genotype and oral cancer risk\textsuperscript{176}. Another American based study neither found associations nor gene-environment interactions between \textit{GSTM1} null, \textit{GSTT1} null and \textit{GSTP1} genotypes and the head and neck cancer risk, except for smokers carrying \textit{GSTT1} null genotype\textsuperscript{177}.

Interestingly, even though no overall association between \textit{GSTM1} null, \textit{GSTP1} mutant genotypes and the cancer susceptibility was observed in the study population, the risk was modified by the environmental factors which resulted in significantly increased risk to UADT cancers. Hence, the findings confirm the definitive role of these environmental factors along with the \textit{GST} polymorphisms as risk enhancers in the etiology of UADT cancers in Tamilians. The observations of our study moderately deviate from other Indian studies and other populations. This may be due to the larger sample size of our
study as well as the differences in cultural, linguistic and dietary practices from other populations.

Although the individuals homozygous for *ABCB1* TT genotype had an increased susceptibility to UADT cancers, a significant interaction was seen with increased exposure among regular smokers and tobacco chewers homozygous for TT genotype. In India, the disproportionately higher incidence of UADT cancers in relation to other malignancies may be due to the major risk factors such as tobacco or betel quid chewing, cigarette or bidi smoking, alcohol consumption, low socioeconomic condition related to poor hygiene, poor diet, nutritional deficiencies and viral infections.

The international agency for research on cancer (IARC) confirmed that smoking of various forms of tobacco (e.g., bidis, pipes, cigars and cigarettes) is carcinogenic in humans. The chewing of tobacco with betel quid increases exposure to carcinogenic tobacco-specific nitrosamines. BaP, a PAH, that is found in the tobacco smoke, is a major carcinogen. As P-gp is involved in the transmembrane efflux of PAH and carcinogens derived from tobacco chewing, the present data clearly indicate that the regular smokers or tobacco chewers having TT genotype had a higher carcinogenic risk associated with multiple and progressive genetic aberrations leading to UADT cancers. These findings need to be confirmed in other ethnic groups.

The ROS produced by the oxidation of toxins and carcinogens due to tobacco chewing were found to be significantly high in the saliva. Hence, the transport of carcinogens associated with tobacco consumption could be regulated by the P-gp expressed in the salivary glands. In the study, carcinogens like BaP, 3-MC and PhIP are potent in causing UADT cancers due to the increased frequency of TT genotype. Genetic variability in metabolic activation and detoxification of environmental carcinogens may partially explain host susceptibility to chemically induced cancers. Even though, the
ABCBI 3435TT genotype may confer a substantial risk to UADT cancers among Tamilians, the significant interaction among habitual tobacco smokers and chewers, homozygous for TT genotype modulates the risk of developing UADT cancers in the Tamilian population of south India.

Genetic variations may not cause disease but rather influence a person's susceptibility to environmental factors. By identifying and characterizing polymorphisms of genes involved in the carcinogen activation as well as gene-environment interactions, ultimately, the information can be used to recommend lifestyle modifications in genetically predisposed high risk individuals.

6.4. Association between genotypes and tumor outcome parameters

The known risk factors for poor outcome such as advanced primary tumor size and cervical lymph node metastasis are identified as being significantly associated in the study population. Lymph node involvement is more frequent in patients presenting with late stage disease, but also found in small primary tumors that reflects the aggressiveness of the malignancy. Initial tumor extension is most likely to be influenced by patient behavior because almost all tumors will extend to T4 if diagnosis is delayed.

In our study, the patients were selected within eight months of disease occurrence, to avoid possible disparity associated with the outcome measures among the subjects.

In the study, a significant association was observed between T3/T4 tumors and the polymorphic genotypes of CYP1A1*2A besides predisposing as a strong risk factor in the initiation of UADT carcinogenesis as evidenced by the significant gene-gene and gene-environment interactions. The linear increase in the risk for the heterozygous and homozygous genotypes associated with the intermediate and higher levels of enzyme activity could likely reflect the influence of these mutant genotypes on advanced stage...
tumors. Further, the analysis based on the carcinoma sites demonstrated an association between T3/T4 lesions and pharyngeal cancer among the carriers of both *1A/*2A and *2A/*2A; whereas the oral cavity and laryngeal cancers only approached significance (P=0.08). The data of our study emphasizes the important role of CYP1A1*2A polymorphisms in the metabolic activation of various carcinogens especially among tobacco chewers as evidenced by gene-environment interactions. The mechanism for the association of polymorphic CYP1A1*2A genotypes presumably reflects their influence on the detoxification of potential carcinogens, a key factor in UADT cancers. The findings of a German based Caucasian study did not find a correlation between tumor extension, presence of nodes or histological differentiation with variant alleles of CYP1A1*2A genotype. While the deficient GSTT1 was significantly associated with T3/T4 lesions among the oral cavity and pharyngeal cancers and a significant trend for histological differentiation in the oral cavity and pharyngeal cancers but not in laryngeal cancers was observed291. The data also suggest that the rare genotype of the ABCB1 is associated with the cervical lymph node metastasis.

The association observed for the nodal status was significant among pharyngeal cancers while the cancers of oral cavity only approached significance. A study had demonstrated an association with Pgp-positive tumors and the affected lymph nodes than those with Pgp-negative tumors in the head and neck squamous cell carcinoma patients292. In a German study, the polymorphic CYP2D6*4, CYP2D6*3 and CYP2D6*5 were associated with lymph node involvement in the laryngeal cancer, but neither among oral or pharyngeal cases nor associated with primary lesions and histological differentiation291. The two Chinese studies have shown that the P-gp expression was correlated to the positive expression of c-erbB-2 with the lymph node metastasis in clinical III-IV stage patients of esophageal carcinoma293 and gastric carcinoma294. However, when the c-erbB-2 expression was negative, the lymph node metastasis and clinical staging were not related to the P-gp expression in esophageal as well as in
gastric carcinoma patients. In a breast cancer study, P-gp and multidrug resistance related protein (MRP1) were expressed in lymph node metastasis and MRP1 expression is more pronounced in lymph node metastasis than in corresponding primary tumors. While another study did not find a positive association with P-gp and multidrug resistance related protein (MRP) expression in lymph node metastasis in the carcinoma of breast. The findings of the study suggest that the homozygous mutant genotype of ABCB1 is not only a strong predisposing risk factor for the UADT cancers, but also a genetic marker in the cervical lymph node metastasis in the population. Therefore, this finding would be of clinical significance, if similar studies are replicated among other ethnic groups.

6.5. Association between genotypes and remission and recurrence related to UADT cancers
Recurrent UADT cancers in previously irradiated areas are a severe clinical entity. To the best of our knowledge, this is the first molecular epidemiologic study conducted in India to investigate the association between genetic polymorphisms in cancer susceptible genes and risk of UADT cancer recurrence. It was noted that patients with T3/T4 tumors were more likely to experience a recurrence, providing some assurance that the study findings were consistent with well-documented clinical observations. Similarly, the patients showing lymph node metastasis developed local recurrences more frequently than patients in the absence of cervical lymph nodal status (P= 0.01 & 0.03 respectively; data not shown). In this study, cases who inherited homozygous mutant genotype of CYP1A1*2A tend to play a significant role in the increased risk associated with cancer recurrence when compared to the wild type genotype. Furthermore, the Kaplan-Meier survival function and Cox regression analyses also showed that the individuals homozygous for CYP1A1*2A mutant genotype had a shorter recurrence-free survival. Our observation of poor recurrence free survival for the
CYP1A1*2A variant is supported by a data showing a worse disease outcome for CYP1A1*2A in children with acute lymphoblastic leukemia. A breast cancer study did not observe a role of CYP1A1*2A in the disease outcome, but they demonstrated a significant association of CYP1A1*2C in shorter disease free survival. The polymorphisms in the CYP1A1 gene enhances the activity and inducibility of the enzyme. The enhanced enzyme activity of the polymorphic CYP1A1*2A leading to increased production of the reactive intermediates consequently resulted in poor clinical outcome as evidenced with gene-gene and gene-environment interactions and its role in primary tumor extension. Nevertheless, more studies elucidating genotype-phenotype association using CYP1A1 substrates and cytotoxicity assays are needed to confirm this finding.

The results of our study suggest that many of the patients carrying CYP1A1*2A*2A genotype would not have developed the UADT cancers, if they had not consumed large quantities of tobacco products. This was also confirmed by the recurrence of the cancer noticed in a few patients who had consumed tobacco following the remission. It is likely that the impact on outcome arise through the interplay of gene-environment interactions which predispose an individual to initial cancer development. In this regard, it is indeed plausible that individuals with this variant, particularly in the presence of substrates and/or inducers, not only have an enhanced susceptibility to develop UADT cancers but also recurrent malignancy.