4.1: DISCUSSION

Gene polymorphisms are the basis for the huge diversity in the human phenotype, and thus the huge range of sensitivity to common as well as complex diseases, especially cancer. It is generally accepted that genomic instability is an important indicator of malignancy. Cancer is a disease of disordered gene expression due to mutations within genes or closely linked DNA that regulates the activity of those genes. By studying gene polymorphisms it is expected to reveal which changes are likely to result in disease. Single nucleotide polymorphisms (SNPs) that harbor coding and non-coding regions of a gene have the potential to change protein function or its expression and led directly to cause variations in the expression of disease phenotype.

The role of Cytochrome P450 (CYP) superfamily and its genetic determinants in the metabolism of drugs and endogenous compounds have long been suspected [377-379]. It is well known that the induction of CYP1A1 plays a major role in the development of oral cancer and other smoking-related respiratory tract cancer [378]. PAHs present in tobacco smoke activates transcription of the CYP1A1 gene [380] and CYP1A1 enzyme converts the PAHs into active carcinogens.
There are contradictory reports on the role of *CYP1A1* variants in altering the level of gene expression or the mRNA stability. The Ile462Val polymorphism exhibited increased levels of induced or basal *CYP1A1* mRNA with increase in Val variants, while in purified *Escherichia coli*, Ile and Val variants did not exhibit differences in the metabolism of benzo[a]pyrene [89, 96]. In the present study, investigation of IVS1-728G>A (rs4646421), Thr461Asn (rs1799814) and Ile462Val (rs1048943) at the gene of *CYP1A1* revealed only IVS1-728G>A and Ile462Val are polymorphic in the South Indian population. Although smoking was established as a risk factor in the present study, IVS1-728G>A and Ile462Val polymorphisms of *CYP1A1* gene was not associated with risk of oral cancer. The SNPs of *CYP1A1* was not associated with oral cancer either at genotype or haplotype level.

The Thr461Asn (m4) is specific for caucasians with a frequency ranging from 3% to 6%, has also been related to greater enzyme catalytic efficiency [382], none of the subjects in our sample carried this variant. The famous and well studied Ile462Val (m2) in *CYP1A1* is present in both Caucasians (7%) and Asians (35%) and is mostly detected in linkage disequilibrium with m1 (MspI site) [383]. A study from North India showed 51% and 17% of *CYP1A1* m2 allele (462Val carriers) in oral cancers normal controls respectively and
associated with an increased risk of oral cancer [384]. In another study from North Indian population the frequencies of genotypes were similar in patient and control groups and failed to show a significant association [385]. Analysis of Caucasian oral cancer patients revealed almost identical heterozygous genotype distributions between cases and controls (6.5% oral cancer 4.3% controls) and failed to show an association between m2 and oral cancer [386]. The Ile462Val heterozygous genotype is significantly associated with earlier age of onset OSCC than that in homozygous wild genotype [387]. A population based case-control study from Puerto Rico, revealed that the genotypes of CYP1A1 (462Val) variant were not associated with the oral cancer risk [388].

Several studies reported lack of association between CYP1A1 genotypes and oral cancer from different geographic regions such as Japan [389] Brazil [390-392], USA (using whites) [393] Jakarta [394]. In contrast to this CYP1A1 (m2/m2) genotype showed association with increased risk of OSCC in Koreans [395]. Furthermore, gene to gene interaction studies showed, GSTM1 null and CYP1A1 polymorphism increased the risk of head and neck cancer [172] and oral cancer [396].
CYP2E1 is present in the brain and plays a major role in both nicotine and ethanol metabolism. Systematic investigation of three CYP2E1 gene SNPs (V179I, Rsal and Pstl) in 132 oral cancer patients and 157 controls revealed that the V179I is not polymorphic and Rsal/Pstl is not associated with oral cancer either at genotype or haplotype level. The mutant allele frequency of the CYP2E1 gene is higher in the controls (2.7%) than the oral cancer patients (1.9%). Previous studies suggested that the “A” allele V179I locus was observed only in African and European populations [397, 398]. Analysis of French oral and pharyngeal cancer patients showed an increased risk for oral or pharyngeal cancer in the carriers of CYP2E1 c2 allele amongst the heaviest drinkers [399]. Screening of 570 Caucasians and African Americans revealed two new alleles, c3 (Rsal [+] /Pstl [+]) and c4 (Rsal [-] /Pstl [-]), but their frequency is much less. In both Caucasians and African Americans the c1 allele is associated with oral cancer in subjects who smoked less-than or equal-to 24 pack-years (P=0.033) but not in the heavy-smoking group (i.e. > 24 pack-years) [400]. In contrast to this very low frequency of CYP2E1 Rsal variant in the Greek population, this SNP cannot have an important effect on oral cancer risk [401]. Screening of CYP2E1 Pstl, Rsal and Dral polymorphisms in one Indian population revealed similar genotype frequencies in leukoplakia and controls groups [385]. A strong correlation
between c2 allele of CYP2E1 and high frequency of safrole-DNA adducts in the peripheral white blood cells of areca quid chewing individuals demonstrated the CYP2E1 mediated modulation of safrole-DNA adduct formation [402]. Evaluation of 10 genetic polymorphisms of nine genes in Japanese oral squamous cell carcinoma (OSCC), found that CYP2E1 polymorphisms significantly affected the OSCC risk [389]. A hospital-based case-control study from Brazil demonstrated that the CYP2E1-PstI mutant allele increased the risk for oral cancer [241], but another study from the same region failed to demonstrate significant association between CYP2E1 polymorphisms and oral cancer [390]. There were no significant differences between oral cancer and control groups for CYP2E1*1B, CYP2E1*5B and CYP2E1*6 polymorphisms when analyzed separately, but the gene–environment interactions analyses revealed significant interactions among tobacco smokers, regular tobacco chewers and alcoholics carrying CYP2E1*1B mutant genotypes. This indicates CYP2E1 genotypes may confer a substantial risk for upper aerodigestive tract cancers among Indians [403]. An analysis of the gene variation in eight metabolic enzymes revealed that the CYP2E1 is not associated with oral cancer in Caucasians. [393]
It is difficult to obtain more robust conclusions about the role of CYP2E1 genetic variations in the susceptibility of oral cancer because previous reports of CYP2E1 SNP associations with an increased OC risk provide conflicting evidence. Meta-analysis of 21 case control studies using PstI/Rsal polymorphism of CYP2E1, revealed a significantly high cancer risk for the c2 homozygote in Asian populations, but not in Caucasian populations under any of the three genetic models analysed [404]. The differences that have been observed may be due to the differential distribution of less common c2 allele between various races (Caucasians 5-10%; Asians ~25-50%) [397, 398, 405]. The lack of a significant association between CYP2E1 gene polymorphisms and oral cancer in the present study might be explained by the substantially lower frequencies of CYP2E1 c2 allele.

Systematic investigation of three functional SNPs (rs1051740, rs2292566 and rs2234922) at the EPHX1 gene in the south Indian population showed that SNPs of EPHX1 was not associated with oral cancer either at genotype or haplotype level. In the present study all three EPHX1 gene polymorphisms have shown similar minor allele frequencies in controls and cases. As the allele frequencies were similar in both cases and controls the main effects of the EPHX1 genotypes was not seen in many studies [255, 406, 407].
Analysis of squamous cell carcinoma of the head and neck for Tyr113His and His139Arg loci has shown similar minor allele frequency in both case and controls (Tyr113His: case 32.9% and control 28.0%), (His139Arg: case 17.4% and control 20.4%) but the Tyr113His locus deviated significantly for HWE in both case and control groups [408].

Since the identification of EPHX1 functional polymorphisms, a large number of studies were conducted to study the association between EPHX1 genotypes and several cancers. A population-based, case-control study reported a consistent, but statistically non-significant association between low mEH activity and the cancers related to smoking [409]. The predicted mEH activity allele distribution was shown to be significant between control and oropharyngeal cancer patients ($P = 0.03$) or larynx cancer patients ($P = 0.01$) [256]. The exon 3 polymorphism of the EPHX1 gene was associated with a significantly decreased risk of lung cancer [253]. Modifying effect of polymorphisms in an EPHX1 gene on the risk of early-onset lung cancer was also reported [410]. A significant association between predicted high mEH activity genotypes and orolaryngeal cancer risk was observed in Caucasian subjects [255]. Analysis of EPHX1 gene polymorphisms in oropharyngeal cancer in whites did not show any evidence of association [393]. The distribution of Tyr113 allele occurs
more frequently among Africans, Europeans and Americans [411-413] and the Arg139 allele frequency is more in Asian populations than the Caucasians [414, 415]. Lack of significant linkage disequilibrium between rs1051740, and rs2234922 loci indicated that these polymorphisms are predicted to exist in separate haplotype blocks [416]. However, the present study did not show a significant association between the EPHX1 genotype and oral cancer in our population.

Systematic investigation of three NAT2 gene functional SNPs (rs1799929, rs1799930 and rs1799931) in 132 oral cancer patients and 157 controls, both of South Indian origin, showed that these SNPs of NAT2 is not associated with oral cancer either at genotype or haplotype level. Furthermore, the NAT2 individual deduced acetylator phenotypes have not yielded statistically significant ORs, when calculated in relation to NAT2*4/*4 as reference genotype. But the overall acetylator genotypes (deduced phenotype based on NAT2 allele nomenclature), showed statistically significant association, in particular rapid acetylator genotypes, to oral cancer risk. Approximately 55 years ago, the differences in response to isoniazid toxicity in patients with tuberculosis led to the identification of NAT2 acetylation polymorphism [417]. This polymorphism was known as “isoniazid acetylation polymorphism” until its Pharmacogenetics was fully
Gene polymorphism and risk of oral cancer in a South Indian population – a case control study

comprehended [418]. Since the identification of NAT2 functional polymorphisms, a large number of studies have been conducted to study the association between NAT2 genotypes and several cancers [119, 323, 419-421].

Most of the initial studies have not distinguished OSCC from other HNSCC and yielded conflicting results [422-424]. Furthermore, the studies with clearly defined phenotype of OSCC in relation to NAT2 genotype have not produced consistent results [425-427]. The data from two King and Snohomish population-based study with 341 cases and 552 controls failed to show an overall association between acetylator status with OSCC risk; the odds ratios for slow and intermediate acetylators, as compared with the rapid acetylators, were 1.2 (95% CI 0.7–2.2) and 1.1 (95% CI 0.6–2.0), respectively [428]. Almost identical genotype distributions between German Caucasian cases and controls were observed for all three NAT2 acetylators [386]. A case-control study from Brazil suggests that NAT2 polymorphism, alone or combined with GSTM3, may modulate susceptibility to oral cancer [390]. Analysis of NAT2 polymorphisms for squamous cell carcinoma of the head and neck (HNSCC) also revealed a lack of interaction between the polymorphisms and the environmental exposures suggest that chronic consumption of tobacco and alcohol overwhelm enzyme defenses, irrespective of genotype.
Analysis of variation in the genes of eight metabolic enzymes revealed that the \textit{NAT2} fast acetylators were overrepresented in cases (53.7\%) compared with controls (43.9\%) (p value=0.03) indicating that the fast \textit{NAT2} acetylation as a risk factor for oral cancer in whites [393]. A largest genetic epidemiologic study on upper aerodigestive tract (UADT) cancers in Europe that analysed 554 SNPs from 92 genes in the subjects from 14 centers within 10 European countries failed to demonstrate the association of \textit{NAT2} with oral cancer [429]. Similar results were observed in relation to cancers of the upper aerodigestive tract, including oral cavity, pharynx, larynx and esophagus, in northern Italy [32]. However, the present study did not show a significant association between the \textit{NAT2} slow acetylator genotype and oral cancer in our population.

Identifying common genetic variants that involved in the disease risk is possible by studying polymorphisms of the candidate genes using association studies. It is well known that the true associations between gene polymorphisms and disease can be established by using the candidate gene approach in well defined populations. Studies using the gene polymorphisms are not always replicated in other studies consequently may not yield a clear understanding about the causative role of the identified genes. In general many gene
polymorphisms linked with complex diseases contribute only a modest effect on disease risk, therefore it is also important to consider gene to gene and gene with environment interactions in studying the disease risk.