RESEARCH COMMUNICATION

Genetic Polymorphisms of the *CYP2E1* Gene do not Contribute to Oral Cancer Susceptibility in South Indians

Lakshmi Balaji[1], Krishna Balaji Singh[2], LVKS Bhaskar[3*]

Abstract

Cytochrome P450 (CYP) is a super family of mixed-function oxidases that are responsible for the human metabolism of drugs and endogenous compounds, as well as environmental and dietary substances. Many CYP enzymes function in the liver, but presence of *CYP2E1* in the brain is demonstrating its role in both nicotine and ethanol metabolism. To examine the association between *CYP2E1* polymorphism and the risk of oral cancer, we performed a case-control study on a south Indian population. 157 patients with oral cancer and 132 age and sexmatched controls were recruited. Three SNPs of the CYP2E1 gene [4768G>A (p. V179I, dbSNP rs6413419), *CYP2E1*_-1295G>C (dbSNP rs3813867) and *CYP2E1*_-1055C>T (dbSNP rs2031920)] were genotyped using TaqMan allelic discrimination. The V179I locus is monomorphic in the study subjects, whereas rs3813867 and rs2031920 are co-inherited with a minor allele frequency of 0.022. None of the polymorphic sites deviated from HWE in controls. A much lesser frequency of the uncommon c2 allele was seen in our control subjects than in Caucasians and East Asians. There were no significant differences between oral cancer and controls in the distribution of either allelic or genotype frequencies. None of the haplotypes showed a significant association with oral cancer. Our results suggest that *CYP2E1* is not a major or independent determinant in the pathogenesis of oral cancer in south Indians.

Keywords: CYP2E1 - haplotypes - alleles - oral cancer

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Introduction

Oral cancer is the 3rd most common cancer in India after cervical and breast cancer (Jhavar et al., 2004). The most common risk factors for the development of oral cavity cancer are the use of tobacco and alcohol. Polymorphisms in genes involved in the metabolism of carcinogens contained in tobacco and alcohol have been linked to individual susceptibility to oral cancer. Cytochrome P450 (CYP) is a superfamily of mixed-function oxidases that are responsible for the human metabolism of drugs and endogenous compounds, as well as environmental and dietary substances. Many CYP enzymes function in the liver, but presence of *CYP2E1* in the brain is demonstrating its role in both nicotine and ethanol metabolism. A two-to-three-fold increase in *CYP2E1* expression in multiple regions of the brain after introducing ethanol or nicotine indicates induction of *CYP2E1* upon their administration (Howard et al., 2003).

The CYPs are regulated not only directly by nicotine and ethanol but also indirectly via an increase in the ethanol consumption in the presence of nicotine pre-treatment (Yue et al., 2009). When alcohol consumption is high, the *CYP2E1* catalyzes ethanol into acetaldehyde and produces reactive oxygen species (ROS) and N-nitrosamines (Guengerich et al., 1991; Seitz and Stickel, 2007). N-nitrosamines are formed endogenously in the stomach and are present in various environmental factors including tobacco smoke (Mueller et al., 1986). The *CYP2E1* gene is mapped to chromosome 10q24.3-qter. The gene spans over 11 kb and contains 9 exons coding for a membrane-bound protein consisting of 493 amino acid residues with a molecular weight of ~ 57 kDa (Lewis et al., 1997). Both the 5'-flanking region (5'-FR) and 3'-untranslated-region (3'-UTR) harbour several mutations known to alter the transcriptional activity of the gene (Chen et al., 2006; Hayashi et al., 1991).

Two point mutations in the 5'-FR PstI and RsaI- which are in close linkage disequilibrium- are known to generate the *CYP2E1*_1 (c1) allele and the less common *CYP2E1*_2 (c2) allele. PstI and RsaI have been associated with a greater risk for oral, pharyngeal (Gattas et al., 2006; Matthias et al., 2002), liver (Hata et al., 2010; Ye et al., 2010) and lung cancers (Klinchid et al., 2009; Wang et al., 2010). The rare c2 allele frequency constitutes 24–30% for East Asian populations (Kato et al., 1992; Tan et al., 2000), 2–3% for Caucasians (Kato et al., 1992; Carriere et al., 1996), 0.3–7% for Afro-Americans (London et al., 1996; Wu et al., 1997), 15% for Mexican Americans (Wu et al.,

[1]*Department of Endodontics,* [2]*Department of General Surgery, Sri Ramachandra Medical College and Hospital,* [3]*Department of Biomedical Sciences, Sri Ramachandra University, Chennai, India *For correspondence: lvksbhaskar@gmail.com*

Table 1. Primers and Probes used for Genotyping *CYP2E1* Gene Polymorphisms

CSNP	Primers/Probe	Sequence
rs6413419	C__30443971_10¶	
rs3813867	C__2431875_10	
rs2031920	Forward	TGACTTTTATTTTCTTCATTTCTCATCATATTTTCTATTATACAT
	Reverse	GTTTTTCATTCTGTCTTCTAACTGGCAATAT
	Probe 1 (VIC)†	AGGTTGCAATTTTGTACTTT‡
	Probe 2 (FAM)	AGGTTGCAATTTTATACTTT

¶Probes and primers were obtained as 'Pre-designed SNP genotyping assays' and vendor (Applied Biosystems) did not provide sequence information. The assay numbers are those given by the suppliers. †Probes corresponding to different alleles were labeled with VIC and FAM fluorescent dyes (Applied Biosystems). ‡Polymorphic bases are underlined.

1997) and 18% for Taiwanese (Hildesheim et al., 1995). The present study was aimed to investigate the association between oral cancer and three sequence variations that are reported to result in impaired transcription of *CYP2E1* gene.

Materials and Methods

Subjects

For the present study, samples were collected from a relatively homogenous Tamil population from Tamil Nadu, India. Oral cancer patients were recruited from the Kanchipuram Cancer Hospital in the suburban town of Kanchipuram. Control samples were collected from Sri Ramachandra Dental College in Chennai and broadly represent matched ethnic and socio-economic backgrounds with that of the cases. The present study comprises 157 oral cancer patients, all confirmed by histopathology to have squamous cell carcinoma, and 132 control subjects who reported the absences of personal history of any type of cancer. For cases and controls, the information regarding age, gender, occupation and details about duration, frequency, nature of tobacco habit (smoking or smokeless) and alcohol consumption were noted through a detailed questionnaire. Controls enrolled in this study were matched for age, gender and tobacco habits. All the patients who participated in the study gave informed written consent prior to the study. This study was approved by Sri Ramachandra University Review Committee for Protection of Research Risks to Humans. Peripheral blood samples (3–5 ml) were collected in EDTA coated vacutainers from all the participants.

Genotyping

DNA was isolated from the above samples following the Sambrook et al. (Sambrook et al., 1989) protocol. Three SNPs of the *CYP2E1* gene [4768G>A (p. V179I, dbSNP rs6413419), *CYP2E1*_-1295G>C (dbSNP rs3813867) and *CYP2E1*_-1055C>T (dbSNP rs2031920)] were genotyped. The primers and probes for all the SNPs used in this study were purchased from Applied Biosystems, Foster City, CA, USA (Table 1). PCR amplification was performed using Applied Biosystems' TaqMan 7900HT Fast Real-Time PCR System, and fluorescence was also measured using its sequence detection software (SDS), version 2.3. Each reaction contained 2.5 μL TaqMan Universal PCR Master Mix, 0.125 μL TaqMan SNP Genotyping Assay, 1.375 μL distilled water and 1 μL DNA (10 ng/μL), for a final reaction volume of 5 μL. The analysis was carried out in 384-well optical reaction microplates (Applied Biosystem). PCR was performed by an initial activation of AmpliTaq gold (95oC for 10 minutes) followed by 40 cycles of denaturing (95oC for 15 seconds) and annealing/ extension (60oC for 1 minute). The plate also contained at least two no-template controls without any DNA.

Statistical analysis

Allele frequencies were calculated by gene counting method for the case and control samples independently. The genotype distribution for each site in each sample was evaluated for Hardy–Weinberg equilibrium. The observed and expected genotype distributions and allele frequencies were computed using the HWSIM program (Cubells et al., 1997). The strength of the association of oral cancer and controls between *CYP2E1* gene polymorphisms in three genetic models (additive, dominant, and recessive) was evaluated using the odds ratio and the $\chi 2$ test. For the computation of percentages, odds ratios (OR) with 95% confidence interval and chi square tests, we used the statistical package SPSS 14.0. Linkage disequilibrium (LD) values of D' and r2 were estimated using HaploView 3.12 (Barrett et al., 2005). To perform haplotype-phenotype analysis we have used the THESIAS program (www.genecanvas.org).

Results

The present study includes 54.8% and 34.8% men in oral cancer and control groups respectively. The mean age of the control group was 53.1±10.7 years, and 55.1±10.6years for the oral cancer group. There was no significant difference between the control and cancer groups (p=0.113).

Out of three SNPs analysed only two were polymorphic with minor allele frequency of 0.022 for both rs3813867 and rs2031920. The two SNPs [(*CYP2E1*_-1295G>C (dbSNP rs3813867) and *CYP2E1*_-1055C>T (dbSNP rs2031920)] found in the *CYP2E1* gene co-inherit in the Indian population also. The genotype frequencies of the homozygotes for wild type allele (*a), heterozygotes (*a/*b) and homozygotes for the rarer mutant allele (*b) in the oral cancer were observed to be 151 (96.18%), 6 (3.82%) and 0 (0.0%), respectively, as compared to 125 (94.70%), 7 (5.30%), and 0 (0.0%) in controls (Table 2). The rs6413419 was monomorphic in both cases and controls. None of the polymorphic site deviated from HWE. There were no significant differences in genotype or allele frequencies between controls and cases with oral

100.0
6.3
75.0
56.3
50.0
25.0
31.3
0
Newly diagnosed without treatment

Table 2. *CYP2E1* Gene Polymorphisms and Oral Cancers in Three Different Models

Genotype		Control	Oral cancer	MAF*	OR (P value)		
				case/control	Dominant	Recessive	Additive
rs3813867	CC*2	0 (0.0%)	0 (0.0%)	1.9/2.7	0.710 (0.545)	-	0.715 (0.550)
	CG	7 (5.30%)	6 (3.82%)				
	GG*1	125 (94.70%)	151 (96.18%)				
	HWE p	0.754	0.807				
rs2031920	TT*2	0 (0.0%)	0 (0.0%)	1.9/2.7	0.710 (0.545)	-	0.715 (0.550)
	CT	7 (5.30%)	6 (3.82%)				
	CC*1	125 (94.70%)	151 (96.18%)				
	HWE p	0.754	0.807				

Genotype distribution of the CYP2E mutations; (*1) wild type (rs3813867, and rs2031920) and (*2) mutant allele. * Minor allele frequency; HWE:Hardy-Weinberg equilibrium.

Table 3. *CYP2E1* Gene Haplotypes and Oral Cancer

	rs3813867 and rs2031920			
Halpotype	Control	Case	OR (95% CI)	P value
GC	0.973	0.981	Reference	
CT	0.027	0.019	0.710 (0.232 - 2.166)	0.547

cancer (Table 2). The pairwise LD values (D'=1 and r2=1) between rs3813867 and rs2031920 also revealed that one SNP can act as a surrogate for another. Haplotype analysis using two polymorphic SNPs are provided in table 3. None of the haplotype showed significant association with oral cancer.

Discussion

The role of Cytochrome P450 (CYP) superfamily and its genetic determinants in the metabolism of drugs and endogenous compounds has long been suspected (Lieber, 1997; Rendic, 2002; Rendic and Guengerich, 2010). *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, RsaI and Pstl) in 132 oral cancer patients and 157 controls revealed that the V179I is not polymorphic and RsaI/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 (Kidd et al., 1998; Lee et al., 2008). 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 (Bouchardy et al., 2000). Screening of 570 Caucasians and African Americans revealed two new alleles, c3 (RsaI[+]/PstI[+]) and c4 (RsaI[-]/PstI[-]), 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) (Liu et al., 2001). In contrast to this very low frequency of *CYP2E1* RsaI variant in the Greek population, this SNP cannot have an important effect on oral cancer risk (Zavras et al., 2002). Screening of *CYP2E1* PstI, RsaI and DraI polymorphisms in one Indian population revealed similar genotypes frequencies in leukoplakia and controls groups (Sikdar et al., 2003). 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 (Liu et al., 2004). Evaluation of 10 genetic polymorphisms of nine genes in Japanese oral squamous cell carcinoma (OSCC), found that *CYP2E1* polymorphisms significantly affected the OSCC risk (Sugimura et al., 2006). A hospital-based case-control study from Brazil demonstrated that the *CYP2E1*-PstI mutant allele increased the risk for oral cancer (Gattas et al., 2006), but another study from the same region failed to demonstrate significant association between *CYP2E1* polymorphisms and oral cancer (Marques et al., 2006). 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 (Soya et al., 2008). An analysis of the gene variation in eight metabolic enzymes revealed that the *CYP2E1* is not associated with oral cancer in Caucasians.(Buch et al., 2008)

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/RsaI 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 (Tang et al., 2010). The differences that have been observed may be due to differential distribution of less common c2 allele between various races (Caucasians 5-10%; Asians ~25-50%) (Garte et al., 2001; Kidd et al., 1998; Lee et al., 2008). 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.

In conclusion, there were no significant differences in *CYP2E1* gene polymorphism were found between south Indian oral cancer patients and controls. However, this study is preliminary in nature and requires a larger

sample to validate the association between *CYP2E1* gene polymorphism and susceptibility to oral cancer.

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