Polymorphisms of CYP1A1, GSTM1 and GSTT1 Loci as the Genetic Predispositions of Oral Cancers and Other Oral Pathologies: Tobacco and Alcohol as Risk Modifiers

Sumana Chatterjee · Soujatya Dhar ·
Bani Sengupta · Ashish Ghosh · Manas De ·
Sumit Roy · Sila Chakrabarti

Received: 9 November 2009 / Accepted: 7 June 2010 / Published online: 25 August 2010
© Association of Clinical Biochemists of India 2010

Abstract Polycyclic aromatic hydrocarbons of tobacco require activation by phase I enzymes, such as cytochrome-P4501A1 (CYP1A1) to become an ultimate carcinogen, which are subjected to detoxification by phase II enzymes, especially glutathione *S*-transferases (GSTs). A study was designed to find whether genetic predisposition are risk modifiers of oral pathologies. The study included 102 cases with Oral Cancers (OCs), 68 cases with nonmalignant pathologies, 100 cases as control group. GSTM1 null genotype was associated with increased risk of OCs but not with benign pathologies. Deleted GSTT1 was associated with all pathologies. Both m1m2 and m2m2 polymorphisms of CYP1A1 were associated with oral pathologies.

Keywords Detoxification · Nonmalignant · Risk modifiers · Oral cancers · Genotype

Introduction

Cancers of the oral cavity rank as the eighth most common cancer among men, being responsible for 3% of the cancers diagnosed in this gender. Mortality rates are substantially lower than incidence rates. According to the World Health Organization (WHO) data, the standardized mortality rate for 2002 was 2.2 deaths per 100,000 population. Oral cancer (OC) is one of the leading cancers in most Asian countries [1]. In another study the prevalence of head and neck cancers was found to be significantly high at 54.48% in the population of North-Eastern India. The prevalence is more in males than females. The increasing number of Head and Neck Cancer cases is a cause of major concern as it is associated with high morbidity and mortality in a sizeable population [2].

Human cancer risk is associated with exposure to exogenous or endogenous carcinogens. This can be modified by genetic variations in metabolic detoxification mechanisms [3]. The environment–gene interaction on carcinogenesis has been well demonstrated by phase I and II enzymes that are involved in the metabolism of carcinogens. Some of these enzymes are polymorphic in genotypes, with corresponding variation in their activities. The phase I enzymes, CYPs activate many environmental procarcinogens by adding or exposing their functional groups [4]. Most polycyclic aromatic hydrocarbons first require activation by phase I enzymes, such as cytochrome P4501A1 (CYP1A1) to become an ultimate carcinogen. These activated forms may be subjected to detoxification by phase II enzymes, especially glutathione *S*-transferases (GSTs) [5]. GSTs play a role in detoxification of a variety of endogenous and exogenous electrophilic compounds, such as the removal of reactive oxygen species and regeneration of *S*-thiolated proteins that are products of

S. Chatterjee · S. Dhar · B. Sengupta · S. Chakrabarti (✉)
Thalassaemia Research Unit, Ramakrishna Mission Seva
Pratishthan, Vivekananda Institute of Medical Sciences, 99,
Sarat Bose Road, Kolkata 700026, West Bengal, India
e-mail: Sheelachakrabarti@gmail.com

A. Ghosh · M. De
Department of Oral and Maxillofacial Surgery, Ramakrishna
Mission Seva Pratishthan, Vivekananda Institute of Medical
Sciences, 99, Sarat Bose Road, Kolkata 700026, West Bengal,
India

S. Roy
Dental Clinic, Ramakrishna Mission Seva Pratishthan,
Vivekananda Institute of Medical Sciences, 99, Sarat Bose Road,
Kolkata 700026, West Bengal, India

oxidative stress and the detoxification of carcinogenic compounds [6]. Eight distinct classes of the soluble cytoplasmic mammalian GST have been identified: alpha, kappa, mu, omega, pi, sigma, theta and zeta [7]. Several studies have shown that *GSTM1* polymorphism is associated with an increased risk of lung, bladder, gastric, colorectal, skin, laryngeal cancer preoperatively or post-operatively [8]. *GSTT1* is involved in the biotransformation of several low molecular-weight toxins, such as methyl-halogenids or ethylene oxide, part of the population lacking this enzyme might be more susceptible to smoking or alcohol-related cancers [9, 10].

The term ‘leukoplakia’ describes a white plaque that does not rub off and cannot be clinically identified as another entity. Most cases of leukoplakia are a hyperkeratotic response to an irritant and are asymptomatic, but about 20% of leukoplakic lesions show evidence of dysplasia or carcinoma at first clinical recognition is sometimes used to indicate a premalignant condition [11]. The premalignant or malignant potential of lichen planus is in dispute. Some believe that the occasional epithelial dysplasia or carcinoma found in patients with this relatively common lesion may be either coincidental or evidence that the initial diagnosis of lichen planus was erroneous [12]. The present study categorizes the different types of oral pathology as cancerous group including frank cancerous lesions and leukoplakias and other oral pathology group that includes traumatic oral ulcers, benign oral lesion; unknown white patches and lichen planus. The aim of the study was to find association of deletions of *GSTM1* and *GSTT1* genotypes and presence of m2 allele of *CYP1A1* gene in the development of OCs and their modification of tobacco and alcohol.

Materials and Methods

Study Cases

The study recruited 170 cases (102 cases with cancerous and precancerous oral lesions and 68 cases with non-malignant oral lesions) attending the outpatient department of Department of Oral and Maxillofacial Surgery and Dental Clinic of Ramakrishna Mission Seva Pratishthan hospital from May, 2007 to March, 2009. Cases with minor oral problems, e.g. stained teeth, oral infection were excluded from the study. Age and sex matched normal control group comprising of 100 individuals with no oral pathology were taken for the study from the respective outpatient departments. All subjects were clinically checked and histologically confirmed. All participating subjects were given questionnaire to have information regarding their life-style, tobacco and alcohol intake and other ailment. Informed

written consent was obtained from all individuals. All the study subjects were Bengali, Indian and belonged to the same ethnic group. The study protocol was approved by the ethical and research advisory committee of the institution.

DNA Polymorphism

An aliquot of blood (2–3 ml) was collected from antecubital vein of the subjects in a 5 ml EDTA vacutainer. DNA was isolated from the peripheral leukocytes using standard salting out method [13]. Fifty to 100 ng of DNA were amplified in a total volume of 25 µl containing 10 mM Tris/HCl pH 9.0, 2 mM MgCl₂, 200 µM of deoxynucleotide triphosphate, 1 µM of each primer, and 0.2 units of *Taq* polymerase.

CYP1A1 Gene

Different polymorphisms of *CYP1A1* were identified by PCR amplification followed by digestion with *MspI* restriction enzyme. The enzyme detects the substitution of CCGG for CTGG in *MspI* site. The PCR cycle involved 30 cycles of the following condition: 1 min denaturation at 95°C; 1 min annealing at 57°C; 1 min primer extension at 72°C. Amplified fragments were digested with 2U *MspI* per reaction for 2–4 h at 37°C. Digested products were electrophoresed in 2% agarose gel (100 V, 45 min). Genotypes of all individuals were noted as m1m1: 1078 bp; m1m2: 1078 bp, 878 bp; 200 bp; m2m2: 878 bp; 200 bp [14, 15].

GST Gene

GSTM1 (220 bp) and *GSTT1* (450 bp) portions were co-amplified with β -globin (268 bp) using a multiplexing PCR programme of 35 cycles with 1 min denaturation at 94°C; 1 min annealing at 62°C; 1 min primer extension at 72°C. Amplified PCR fragments were visualized into an ethidium bromide-stained 2% agarose gel after electrophoresis (100 V, 1.0 h). Absence of 220 and 450 bp portions implied *GSTM1* and *GSTT1* null genotypes, respectively. Subjects with all fragments were non-null genotypes [16].

All the results were checked twice in double blind.

Statistical Analysis

The relationships between OCs and other oral pathologies and relevant risk factors were analysed using OR and their 95% CIs derived from stepwise logistic regression analysis using SPSS version 14.0 software. The categorizations of several forms of tobacco and alcohol intake were based on various published literatures and general distribution among the population controls. Crude ORs were calculated

for every independent variable. From prior knowledge of risk factors for OCs, various confounding factors, e.g. age, sex, tobacco habit etc. were adjusted in logistic regression model when adjusted ORs were calculated. To examine the interaction between environmental and genetic factors, stratification analyses of OC risk associated with *CYP1A1*, *GSTM1* and *GSTT1* were carried out by betel quid chewing and alcohol drinking. In the final multiple logistic regression analysis, age, ethnicity, alcohol drinking, combined *GSTM1/GSTT1* genotypes and a combination of *CYP1A1* genotype and mixed oral habits were included. Differences in genotypic frequencies distribution between diseased and control groups was done using 2×2 contingency χ^2 test. All statistical tests were based on two-tailed probability.

Results

Different demographic characteristics of the subjects are summarized in Table 1. None of the study groups showed significant differences in smoking habit (cigarettes/day) and other life style factors. In each of the study groups the male participants were significantly higher in number in comparison to female ones. The study cases except the healthy controls were addicted to different forms of tobacco and alcohol. 50.04 and 48.53% were the bidi (a dry rolled temburni leaf containing fine tobacco dust) smokers, 82.35% were cigarette smokers, 71.57 and 52.94% were addicted to raw tobacco among the OCs and other pathology study groups, respectively. Majority of the study cases except the healthy controls were addicted to mixed (bidi, cigarette, tobacco etc. together) and other oral habits, e.g. catechu, zarda (flavoured tobacco) etc. The mean of smoking or other oral habits was >7 times/day. Among all the study cases 52.56% were well-employed, 0.23% were daily wage earners, 7.76% were small-scale businessmen and 39.45% were housewives irrespective of age and sex. It was confirmed from the life-style factors that none of the subjects were occupationally exposed to the carcinogens. We did not find any association of educational and economic background of the subjects with the studied pathologies.

The buccal cavity was found to be the principle site for the onset of cancerous lesions among the studied cases followed by tongue, cheek and other portions. Squamous cell carcinoma was the most common histological type. The nonmalignant study cases were mostly affected with benign ulcers and lichen planus (Tables 2, 3). The prevalence of studied genotypes were compared with the cancer cases with different stages (Duke's classification system) of the disease (Table 4). None of the studied cases had the history of recurrence of malignancy at any part of the body. No case died during the period of observation. None of the

studied polymorphisms were associated with the development of OCs of certain histological stages.

The distribution of the *GSTM1*, *GSTT1* and *CYP1A1* genes in the study group with different oral pathology is summarized in Table 5. Differences in studied polymorphisms in the subjects showed that *GSTM1* null genotype was strongly associated with development of OCs and not associated with the other pathology group. Null *GSTT1* gene distribution was significantly associated with OCs (OR: 1.5; CI: 5.47–0.41), showing less association with the other pathology group (OR: 1.10; CI: 5.05–0.24). No association was noted with any kind of pathology in absence of both *GSTM1* and *GSTT1* genes. The presence of both *GSTM1* and *GSTT1* genes was observed less frequently in nonmalignant group as compared to malignant group. Both m1m2 and m2m2 genotypes were found to be strongly associated with the development of all kind of oral pathologies. From all data, it is evident that null genotypes of *GSTM1*, *GSTT1* and m1m2, m2m2 polymorphisms of *CYP1A1* are the risk factors of the OCs as compared to the healthy control group in the studied population.

The interactions among *GSTM1*, *GSTT1* and *CYP1A1* polymorphisms and different forms of tobacco use and alcohol intake with the risks of malignant and nonmalignant oral pathologies are summarized in Tables 6, 7, 8 and 9. In case of *GSTM1* deleted individuals the OR for heavy cigarette smokers (>35 sticks per day) was 3.038 (95% CI: 6.268–1.472), i.e. 5.9 times higher than for moderate smokers (<35 sticks per day) (OR: 0.512; CI: 1.148–0.228). Moderate bidi smoking (<35 sticks per day) was associated with OCs (OR: 11.55; CI: 39.81–3.35) and other pathologies (OR: 21.621; CI: 7.87–5.94). No significant association was found among the *GSTM1* deleted individuals with nonmalignant pathologies with any dose of cigarette smoking and smokeless tobacco intake. *GSTM1* null genotypes present in OCs were significantly associated with smokeless tobacco intake in heavy (>10 times per day) and moderate doses (<10 times per day). On the other hand moderate chewing habit (ten times per day) was found to be more associated than the OC individuals with heavy chewing habits (>10 times per day). In case of *GSTT1* deleted individuals, no association of cigarette smoking was found with any oral pathology. Moderate bidi smoking (<35 sticks per day) was found to be more significantly associated with the development of oral lesions in comparison to heavy bidi smoking (>35 sticks per day) in *GSTT1* null cases, which is found to be contrary to our hypothesis. This result may change if analysed with more cases in this group. Smokeless tobacco intake was found to be associated with OCs in *GSTT1* null cases in both heavy (OR: 1.364; CI: 2.678–0.694) and moderate exposure (OR: 1.535; CI: 3.18–0.741), but not with other pathology. Alcohol intake in all doses was found to be risk factors of

Table 1 Demography of the study population with respect to clinical and nonclinical parameters

	Cancer cases	Nonmalignant cases	Normal control
Age (years)			
Mean $\pm$ SD	58 $\pm$ 12.06	51.0 $\pm$ 11.7	49.0 $\pm$ 9.77
Range	18–81	22–68	16–78
<40	11 (10.78)	3 (4.41)	30 (30)
40–60	21 (20.59)	17 (25)	8 (8)
>60	70 (68.63)	48 (47.06)	62 (62)
χ^2 p	0.199	0.199	
Gender (n, %)			
Male	71 (69.6)	40 (58.8)	69 (69)
Female	41 (40.2)	28 (41.18)	31 (31)
χ^2 p	0.157	0.157	
Educational background (n, %)			
Illiterate	4 (3.92)	2 (2.94)	13 (13)
Less than high school	37 (36.27)	27 (39.7)	9 (9)
High school/college	39 (38.24)	20 (29.4)	38 (38)
More than college	22 (21.57)	19 (27.94)	40 (40)
χ^2 p	0.002	0.451	
Clinical investigation (n, %)			
Frank cancer	91 (89.22)	–	–
Leukoplakia	11 (10.78)	–	–
Lichen planus	–	48 (70.59)	–
Traumatic ulcers	–	13 (19.12)	–
Unknown white patches	–	7 (10.29)	–
Average alcohol intake PW (n, %)			
0	51 (50)	61 (89.71)	78 (78)
<50	35 (34.31)	6 (8.82)	15 (15)
50–100	12 (11.76)	1 (1.47)	5 (5)
>100	4 (3.92)	0 (0)	2 (2)
Average cigarette smoking PW (n, %)			
0	18 (17.65)	12 (17.64)	63 (63)
<35	36 (35.29)	52 (76.47)	28 (28)
>35	48 (47.06)	4 (5.88)	9 (9)
	Cancer cases	Non malignant cases	Healthy control
Average bidi smoking PW (n, %)			
0	53 (51.96)	35 (51.47)	24 (24)
<35	25 (24.51)	27 (39.71)	3 (3)
>35	24 (25.53)	6 (8.82)	10 (10)
Mixed smoking habit (n, %)			
Never	62 (60.78)	55 (80.88)	73 (73)
Exclusive	40 (39.22)	13 (19.12)	27 (27)
Occasional	0 (0)	0 (0)	0 (0)
Chewing habit (n, %)			
Never	91 (89.22)	29 (42.65)	88 (88)
Occasional (mixed chewing dose $\pm$ SD/week)	14 $\pm$ 10.21	15 $\pm$ 9.27	12 $\pm$ 9.66
Mixed oral habit (n, %)			
Never	18 (17.65)	10 (14.7)	28 (28)
Occasional	52 (50.98)	37 (54.41)	46 (46)
Exclusive	32 (31.37)	21 (30.88)	26 (26)
Smokeless/raw tobacco (n, %)			
Never	29 (28.43)	32 (47.06)	35 (35)
<10 PW	42 (41.18)	25 (36.76)	40 (40)
>10 PW	31 (30.39)	11 (16.18)	35 (35)

SD standard deviation, PW per week

Table 2 Clinical information of the cases with oral carcinoma

	No. of patients (<i>N</i> = 102) (<i>n</i> , %)
Site of tumor	
Parotid gland	2 (1.96)
Floor of mouth	3 (2.94)
Tongue	26 (25.49)
Retromolar trigonum	1 (0.98)
Submandibular gland	6 (5.88)
Oral cavity	40 (39.22)
Mucosa cheek	24 (23.53)
Type of malignancy	
Squamous cell carcinoma	69 (67.65)
Verrucous carcinoma	10 (9.80)
Osteosarcoma	2 (1.96)
Leukoplakia	20 (19.61)
Others	1 (0.98)

Table 3 Clinical information of the cases with nonmalignant oral pathologies

	No. of patients (<i>N</i> = 68) (<i>n</i> , %)
Site of tumor	
Oral cavity fibromas	3 (4.41)
Lichen planus (homotropic and heterotropic)	29 (42.65)
Traumatic ulcers	30 (44.12)
Benign white patches	4 (5.88)
Others	2 (2.94)

OCs in *GSTT1* deleted cases but not with other pathology. Chewing betel caused development of other pathology but not OCs in *GSTT1* deleted genotypes. Cigarette and bidi smoking in moderate and heavy amount had significant interactions with *CYP1A1*m1m2 genotypes in the development of OCs but smokeless tobacco showed no interaction. Moderate exposure to alcohol (<50 pegs per week) and chewing (<10 times per day) had associations with OCs and other pathology in m1m2 genotypes of *CYP1A1*. Cigarette smoking caused oral pathologies in individuals with m2m2 genotypes. Bidi smoking in moderate (OR: 13.885; CI: 47.657–4.045) and heavy dose (OR: 15.679; CI: 50.815–4.837) and raw tobacco intake also in moderate (OR: 2.385; CI: 7.457–0.763) and heavy dose (OR: 2.717; CI: 7.009–1.040) were strongly associated with the OCs. Moderate and moderately heavy intake (50–100 pegs per week) of alcohol was associated with OCs in *CYP1A1*m2m2 genotypes. Chewing habits were found to be correlated with oral pathologies in m2m2 genotypes. No association of combined exposure of alcohol and tobacco with varied forms and doses with any oral pathology risk

and studied genotypes by a second stratification analysis (data not shown).

As expected, the combination of studied genotypes with both smoking and drinking alcohol showed increased risks for developing OCs. Stratified with null *GSTM1* and *GSTT1* genotypes to analyze the distributions of *CYP1A1* genotypes in cases and controls, the data in Table 10 shows that there were no significant differences in the respective cases and controls of the different *CYP1A1* genotypes in the absence of *GSTM1* and *GSTT1* genes. There was also not any apparent effect when both *GSTT1* and *GSTM1* genes were absent.

Discussion

Several studies have been undertaken to examine the association among *CYP1A1*, *GSTT1* and *GSTM1* null polymorphisms, as well as oral, pharyngeal and various forms of head and neck cancers in India [16–19]. To the best of our knowledge our study traces out the status of the same correlations even with the nonmalignant oral pathologies along with the OCs in the Bengali population of India. Acting in concert with individual susceptibility, environmental factors such as smoking, diet, and pollutants play a role in most human cancers [11]. Our study reveals the comparison of polymorphisms of certain genotypes in different type of oral pathology. Several reports have been published ascertaining the positive and negative correlations of the studied genetic polymorphisms in the carcinogenesis of oral and head and neck cancers.

Extensive consumption of tobacco and/or alcohol is regarded as risk factors for head and neck cancer. Cigarette smoke contains several (pre)carcinogenic compounds [20]. Recent studies have revealed mutagenic effects of alcohol on human chromosomes in vitro [21]. Betel quid chewing, cigarette smoking and alcohol drinking are the major risk factors for OC in Taiwan. In this study, the three habits all increased the risk of developing OC [21]. The ORs were somewhat similar or even more in some cases to those reported previously [22, 23]. We found that only extensive (>35 sticks per day) cigarette smoking induces the susceptibility of OCs having no effects on NMPs. As per cigarette smoking is concerned our results partially support some of the previous works [21, 22, 24]. Intake of alcohol was found to be strongly associated with every kind of oral pathologies (Tables 6, 7, 8). Some study support our data [15, 17, 25] and others do not [16, 26].

Most chemical carcinogens in environment are procarcinogens and aromatic hydrocarbons. They need to be activated to reactive electrophilic forms by type II metabolic enzymes like CYP450s by initiating the carcinogenesis. Again the reactive electrophilic forms of carcinogen

Table 4 Prevalence of GST and CYP1A1 genotypes in different histological stages of the carcinoma with respect to the control group

Stages	GST polymorphisms							
	Cases				Control			
	M1– (%)	T1 (%)	M1–T1– (%)	M1+T1+ (%)	M1– (%)	T1 (%)	M1–T1– (%)	M1+T1+ (%)
I ^a /LP ^b	27 (26.47)	4 (3.93)	2 (1.96)	20 (19.61)	21 (21)	4 (4)	1 (1)	74 (74)
II ^a	12 (11.76)	1 (0.98)	1 (0.98)	7 (6.86)				
III ^a	11 (10.78)	1 (0.98)	0 (0)	11 (10.78)				
IV ^a	3 (2.94)	0 (0)	1 (0.98)	1 (0.98)				
Total	53 (51.95)	6 (5.88)	4 (3.92)	39 (38.23)				

Stages	CYP1A1 polymorphisms					
	Cases			Control		
	m1m1 (%)	m1m2 (%)	m2m2 (%)	m1m1 (%)	m1m2 (%)	m2m2 (%)
I ^a /LP ^b	9 (8.82)	27 (26.47)	7 (6.86)	49 (49)	32 (32)	19 (19)
II ^a	10 (9.80)	13 (12.75)	8 (7.84)			
III ^a	8 (7.84)	5 (4.90)	10 (9.80)			
IV ^a	3 (2.94)	1 (0.98)	1 (0.98)			
Total	30 (29.4)	46 (45.1)	26 (25.84)			

^a *P* (two sided): 0.157 (Pearson's χ^2)^b Leukoplakias**Table 5** Risk estimates of studied GST and CYP1A1 polymorphisms

Genetic polymorphisms	Malignant group			Nonmalignant group		
	OR (crude)	95% CI	<i>P</i> ^a	OR (Crude)	95% CI	<i>P</i> ^a
<i>Dichotomized</i>						
GSTM1/GSTT1						
GSTM1+	1.0			1.0		
GSTM1–	4.06	1.75–7.54	0.00	0.32	0.307–0.631	0.057
GSTT1+	1.0			1.0		
GSTT1–	1.5	0.41–5.47	0.661	1.10	0.24–5.05	0.896
GSTM1+/GSTT1+	1.0			1.0		
GSTM1–/GSTT1–	0.132	0.024–1.22	0.074	0.216	0.218–2.471	0.019
CYP1A1						
m1m1	1.0			1.0		
m1m2	2.33	1.23–4.44	0.009	1.73	0.852–3.53	0.132
m2m2	2.23	0.212–0.943	0.035	1.83	0.242–1.239	0.148
<i>Trichotomized</i>						
GSTM1/GSTT1						
Nonnull/nonnull	1.0			1.0		
Nonnull/null	4.598	2.525–8.371	0.00	3.202	1.667–6.152	0
Null/null	0.132	0.024–1.22	0.074	0.216	0.218–2.471	0.019
CYP1A1						
m1m1	1.0			1.0		
m1m2 + m2m2	0.434	0.243–0.774	0.005	0.657	0.345–1.252	0.202

^a Calculated by χ^2 analysis comparing genotype distributions between case and control subjects

Table 6 Association of GSTM1 genotype with different confounding factors

	Cancer cases					Nonmalignant cases					Healthy control	
	GSTM1+ (n, %)	GSTM1– (n, %)	OR	95% CI	P^a	GSTM1+ (n, %)	GSTM1– (n, %)	OR	95% CI	P^a	GSTM1+ (n, %)	GSTM1– (n, %)
All subjects	49 (48.04)	53 (51.96)	4.06	1.75–7.54		37 (54.41)	31 (45.59)	0.32	0.307–0.631		79 (79)	21 (21)
Sex												
Male	34 (33.33)	37 (36.27)	0.873	0.459–1.661	0.679	28 (41.18)	12 (17.65)	0.690	0.36–1.319	0.261	50 (50)	19 (19)
Female	15 (14.71)	16 (15.68)				9 (13.24)	19 (27.94)				29 (29)	2 (2)
Age (years)												
<40	6 (5.88)	5 (4.90)	0.019	0.008–0.046	0	27 (39.71)	25 (36.76)	0.807	0.324–2.010	0.645	72 (72)	16 (16)
40–60	10 (9.80)	11 (10.78)	2.190	0.937–5.118	0.07	1 (1.47)	3 (4.41)	0.802	0.205–3.137	0.07	5 (5)	4 (4)
>60	33 (32.33)	37 (36.27)	64.03	18.38–223.08	0.058	4 (5.88)	3 (4.41)	3.767	0.908–15.63	0.068	2 (2)	1 (1)
Cigarette smoking												
Never	15 (4.90)	31 (30.39)				29 (42.65)	23 (33.82)				23 (23)	5 (5)
≤35 PD	28 (27.45)	20 (19.61)	0.512	0.228–1.148	0.104	3 (4.41)	1 (1.47)	0.102	0.044–0.234	0.102	0 (0)	9 (9)
>35 PD	16 (15.69)	2 (1.96)	3.038	1.472–6.268	0.003	5 (7.35)	7 (10.29)	0.472	0.132–1.691	0.249	56 (56)	7 (7)
Bidi smoking												
Never	27 (26.47)	48 (47.06)				16 (23.53)	19 (27.94)				68 (68)	19 (19)
<35 PD	20 (19.61)	5 (4.90)	11.55	3.35–39.81	0.0	17 (25.0)	10 (14.71)	2.16	5.94–7.87	0.0	2 (2)	1 (1)
>35 PD	2 (1.96)	2 (1.96)	0.613	0.163–2.3	0.467	4 (5.88)	2 (4.41)	1.915	6.056–0.606	0.269	9 (9)	1 (1)
Smokeless tobacco												
Never	18 (17.65)	11 (10.78)				20 (29.41)	12 (17.64)				25 (25)	10 (10)
≤10 PD	12 (11.76)	30 (29.41)	1.14	0.58–2.26	0.7	8 (11.76)	17 (25.0)	0.71	0.35–1.452	0.348	38 (38)	2 (2)
>10 PD	19 (18.63)	12 (11.76)	1.479	0.72–1.02	0.29	9 (13.24)	2 (4.41)	0.49	0.21–0.97	0.097	16 (16)	9 (9)
Alcohol use												
Never	30 (48.04)	21 (20.59)				33 (48.53)	31 (41.18)				66 (66)	12 (12)
50 PW	17 (16.67)	18 (17.65)	2.936	1.434–6.012	0.003	3 (4.41)	3 (4.41)	0.413	0.147–1.158	0.093	9 (9)	6 (6)
50–100 PW	0 (0)	12 (11.76)	1.7	0.471–6.137	0.418	1 (1.47)	0 (0)	0.279	0.038–2.007	0.213	3 (3)	2 (2)
>100 PW	2 (1.96)	2 (1.96)	2.256	0.395	0.36	0 (0)	0 (0)	*	*	*	1 (1)	1 (1)
Chewing habit												
Never	2 (1.96)	7 (6.86)				29 (42.65)	0 (0)				78 (78)	10 (10)
≤10 PD	13 (12.75)	39 (38.24)	2.327	1.053	0.037	2 (2.94)	23 (33.82)	*	*	*	0 (0)	7 (7)
>10 PD	14 (13.73)	7 (6.80)	0.72	0.37–1.4	0.336	6 (8.82)	8 (11.76)	30.43	2.28–4.06	0.001	1 (1)	4 (4)

PW per week, PD per day, OR odd ratio, CI confidence interval

* No statistics could be done due to absence of positively weighted cases as two of the weighted variables were having null value

^a Calculated by χ^2 analysis comparing genotype distributions among case and control subjects

Table 7 Association of GSTT1 genotype with different confounding factors

	Cancer cases					Nonmalignant cases					Healthy control	
	GSTT1+ (n, %)	GSTT1− (n, %)	OR	95% CI	P ^a	GSTT1+ (n, %)	GSTT1− (n, %)	OR	95% CI	P ^a	GSTT1+ (n, %)	GSTT1− (n, %)
All subjects	96 (94.12)	6 (5.88)	1.5	0.41–5.47	0.661	65 (95.59)	31 (4.41)	1.10	0.24–5.05	0.896	6 (96)	4 (4)
Sex												
Male	66 (64.71)	5 (4.90)	1.028	0.567–1.866	0.927	38 (55.88)	2 (2.94)	0.644	0.339–1.223	0.327	67 (67)	2 (2)
Female	30 (29.41)	1 (0.98)				27 (39.71)	1 (1.47)				29 (29)	2 (2)
Age (years)												
<40	10 (9.8)	1 (0.98)	0.265	0.123–0.569	0.001	2 (2.94)	1 (1.47)	0.126	0.036–0.443	0.001	29 (29)	1 (1)
40–60	18 (17.65)	3 (2.94)	3.557	1.44–8.763	0.006	17 (25)	0 (0)	3.592	1.452–8.882	0.006	6 (6)	2 (2)
>60	68 (66.67)	2 (1.96)	1.323	0.733–2.398	0.353	46 (67.65)	2 (2.94)	1.479	0.764–2.863	0.246	61 (61)	1 (1)
Cigarette smoking												
Never	34 (33.33)	2 (1.96)				50 (7.35)	2 (2.94)				26 (26)	2 (2)
≤35 PD	44 (43.14)	4 (3.92)	0.57	0.158–2.06	0.391	3 (4.41)	1 (1.47)	0.261	0.046–1.468	0.127	8 (8)	1 (1)
>35 PD	18 (17.65)	0 (0)	0.216	0.104–0.449	0.392	12 (17.65)	0 (0)	0.099	0.215–0.45	0.00	62 (62)	1 (1)
Bidi smoking												
Never	52 (50.98)	1 (0.98)				34 (50.0)	1 (1.47)				23 (23)	1 (1)
≤35 PD	24 (25.53)	1 (0.98)	8.864	1.771–4.43	0.008	27 (26.46)	0 (0)	6.909	1.816–2.6	0.005	1 (1)	2 (2)
>35 PD	20 (19.61)	4 (3.92)	1.076	2.634–3.0	0.873	4 (5.88)	2 (2.94)	0.375	1.236–2.34	0.107	9 (9)	1 (1)
Smokeless tobacco												
Never	25 (24.51)	4 (3.92)				31 (45.59)	1 (1.47)				40 (40)	0 (0)
≤10 PD	41 (40.20)	1 (0.98)	1.364	0.694–2.678	0.368	24 (35.29)	1 (1.47)	0.681	0.341–1.359	0.276	40 (40)	0 (0)
>10 PD	30 (29.41)	1 (0.98)	1.535	0.439–3.18	0.248	10 (14.71)	1 (1.47)	0.485	0.114–1.136	0.096	24 (24)	1 (1)
Alcohol use												
Never	48 (47.06)	3 (2.94)				60 (88.24)	1 (1.47)				74 (74)	4 (4)
<50 PW	34 (33.34)	1 (0.98)	3.613	1.787–7.306	0.00	4 (5.88)	2 (2.94)	0.532	0.193–1.418	0.203	15 (15)	0 (0)
50–100 PW	11 (10.08)	1 (0.98)	1.1	1.221–3.679	0.021	0 (0)	0 (0)	0.247	0.028–2.169	0.207	5 (5)	0 (0)
>100 PW	3 (2.94)	1 (0.98)	2.974	0.522–1.6	0.22	0 (0)	1 (1.47)	*	*	*	2 (2)	0 (0)
Chewing habit												
Never	89 (87.25)	2 (1.96)				27 (26.47)	2 (2.94)				87 (87)	1 (1)
≤10 PD	4 (3.92)	0 (0)	0.692	0.189–2.535	0.578	0 (0)	1 (1.47)	8.815	2.485–3.127	0.00	4 (4)	2 (2)
>10 PD	3 (2.94)	4 (3.92)	0.719	0.193–2.677	0.623	18 (26.47)	0 (0)	7.695	2.2–2.752	0.00	5 (5)	1 (1)

PW per week, *PD* per day, *OR* odd ratio, *CI* confidence interval

* No statistics could be done due to absence of positively weighted cases as two of the weighed variables were having null value

^a Calculated by χ^2 analysis comparing genotype distributions among case and control subjects

Table 8 Association of CYP1A1m1m2 genotype with different confounding factors

	CYP1A1m1m1				CYP1A1m1m2					
	Control cases (n, %)	Cancer cases (n, %)	Non malignant cases (n, %)	Control (n, %)	Cancer cases (n, %)	OR, 95% CI	P ^a	Non malignant cases (n, %)	OR, 95% CI	P ^a
All subjects	49 (49)	30 (29.41)	24 (35.29)	32 (32)	46 (45.10)	2.33 (1.23–4.44)	0.009	27 (39.71)	1.73 (0.852–3.53)	0.132
Sex										
Male	41 (41)	28 (27.45)	23 (33.82)	17 (17)	31 (30.39)	1.786 (0.318–3.065)	0.304	17 (25.0)	0.987 (0.591–5.393)	0.982
Female	8 (8)	2 (1.96)	1 (1.47)	15 (15)	15 (14.71)			10 (14.71)		
Age (years)										
<40	16 (16)	1 (0.98)	2 (2.94)	5 (5)	1 (0.98)	0.317 (0.118–0.847)	0.002	1 (1.47)	0.194 (0.054–0.696)	0.012
40–60	5 (5)	17 (16.67)	5 (7.35)	2 (2)	3 (2.92)	5.517 (1.4–2.105)	0.001	5 (7.35)	2.714 (0.942–7.817)	0.064
>60	28 (28)	12 (11.76)	17 (25)	25 (25)	42 (41.18)	0.908 (0.44–1.876)	0.795	21 (30.88)	1.412 (0.64–3.118)	0.393
Cigarette smoking										
Never	32 (52.0)	3 (2.94)	6 (8.22)	15 (15)	7 (6.86)			4 (5.88)		
≤35 PD	4 (4)	7 (6.86)	2 (2.94)	3 (3)	18 (17.65)	6.034 (1.39–2.62)	0.00	1 (1.47)	4.567 (1.01–2.06)	0.0
>35 PD	13 (13)	20 (19.61)	16 (23.53)	14 (14)	21 (20.59)	14.88 (4.66–4.77)	0.00	22 (32.35)	1.974 (0.436–8.94)	0.378
Bidi smoking										
Never	45 (45)	11 (10.78)	7 (10.29)	27 (27)	38 (37.25)			21 (30.88)		
≤35 PD	2 (2)	6 (5.88)	15 (22.06)	11 (11)	7 (6.86)	0.54 (2.06–3.6)	0.197	5 (5.75)	3.119 (1.12–1.78)	0.003
>35 PD	2 (2)	13 (12.75)	2 (2.94)	4 (4)	1 (1.98)	3.476 (1.376–8.781)	0.008	1 (1.47)	1.154 (0.29–4.59)	0.839
Smokeless tobacco										
Never	26 (26)	13 (12.75)	14 (5.88)	13 (13)	10 (9.8)			1 (1.47)		
≤10 PD	11 (11)	6 (5.88)	0 (0)	14 (14)	24 (23.53)	0.886 (0.376–2.086)	0.781	15 (22.06)	0.259 (0.80–0.838)	0.024
>10 PD	12 (12)	11 (10.78)	10 (14.71)	13 (13)	10 (9.8)	0.914 (0.414–2.017)	0.823	1 (1.47)	0.583 (0.256–1.33)	0.200
Alcohol use										
Never	45 (45)	12 (11.76)	17 (25)	26 (26)	23 (22.54)			19 (27.94)		
<50 PW	2 (2)	18 (17.64)	6 (8.82)	4 (4)	19 (18.63)	8.597 (2.2–3.278)	0.00	8 (7.84)	4.126 (1.16–1.463)	0.007
50–100 PW	1 (1)	0 (0)	1 (1.47)	1 (1)	2 (3.92)	0.068 (0.013–0.34)	0.001	0 (0)	0.060 (0.007–0.476)	0.008
>100 PW	1 (1)	0 (0)	0 (0)	1 (1)	0 (0)	*	*	0 (0)	*	*
Chewing habit										
Never	40 (40)	16 (15.68)	12 (17.65)	32 (32)	44 (43.14)			14 (20.59)		
≤10 PD	5 (5)	11 (10.78)	10 (14.71)	0 (0)	2 (3.92)	6.238 (1.873–20.779)	0.003	4 (5.88)	15.004 (4.64–4.842)	0.0
>10 PD	4 (4)	3 (2.94)	9 (13.24)	0 (0)	0 (0)	*	*	9 (13.24)	*	*

PW per week, PD per day, OR odd ratio, CI confidence interval

* No statistics could be done due to absence of positively weighted cases as two of the weighted variables were having null value

^a Calculated by χ^2 analysis comparing genotype distributions among case and control subjects

Table 9 Association of CYP1A1m2m2 genotype with different confounding factors

	CYP1A1m1m1				CYP1A1m2m2					
	Control (n, %)	Cancer cases (n, %)	Non malignant cases (n, %)	Control (n, %)	Cancer cases (n, %)	OR, 95% CI	P ^a	Non malignant cases (n, %)	OR, 95% CI	P ^a
All subjects	49 (49)	30 (29.41)	24 (35.29)	19 (19)	26 (25.49)	2.23 (0.212–0.943)	0.035	17 (25.0)	1.83 (0.242–1.239)	0.148
Sex										
Male	41 (41)	28 (27.45)	23 (33.82)	11 (11)	12 (11.76)	1.114 (0.450–2.758)	0.815	7 (10.29)	1.127 (0.412–3.081)	0.816
Female	8 (8)	2 (1.96)	1 (1.47)	8 (8)	14 (13.73)	10 (14.71)				
Age (years)										
<40	16 (16)	1 (0.98)	2 (2.94)	9 (9)	9 (8.82)	0.261 (0.10–0.678)	0.006	0 (0)	0.10 (0.024–0.421)	0.002
40–60	5 (5)	17 (16.67)	5 (7.35)	1 (1)	1 (0.98)	14.543 (3.97–5.35)	0.00	7 (10.29)	4.125 (1.22–1.392)	0.011
>60	28 (28)	12 (11.76)	17 (25)	9 (9)	16 (15.89)	0.479 (0.233–0.985)	0.045	10 (14.71)	1.01 (0.462–2.207)	0.980
Cigarette smoking										
Never	32 (52.0)	3 (2.94)	6 (8.22)	16 (16)	8 (7.84)			2 (2.94)		
≤35 PD	4 (4)	7 (6.86)	2 (2.94)	2 (2)	11 (10.78)	1.02 (3.237–3.249)	0.00	1 (1.47)	2.609 (0.549–1.240)	0.228
>35 PD	13 (13)	20 (19.61)	16 (23.53)	1 (1)	7 (6.86)	1.38 (4.045–4.7)	0.00	14 (20.59)	2.90 (9.161–9.18)	0.00
Bidi smoking										
Never	45 (45)	11 (10.78)	7 (10.29)	15 (15)	4 (3.92)			7 (10.29)		
≤35 PD	2 (2)	6 (5.88)	15 (22.06)	0 (0)	12 (11.76)	1.385 (4.045–4.77)	0.00	7 (10.29)	0.64 (0.15–2.728)	0.546
>35 PD	2 (2)	13 (12.75)	2 (2.94)	4 (4)	10 (9.80)	1.5679 (4.837–5.08)	0.00	3 (4.41)	1.17 (3.1–4.476)	0.00
Smokeless tobacco										
Never	26 (26)	13 (12.75)	14 (5.88)	4 (4)	10 (9.80)			17 (25)		
≤10 PD	11 (11)	6 (5.88)	0 (0)	15 (15)	12 (11.76)	2.385 (7.457–7.63)	0.135	10 (14.71)	0.148 (0.038–0.573)	0.006
>10 PD	12 (12)	11 (10.78)	10 (14.71)	0 (0)	10 (9.80)	2.717 (1.040–7.009)	0.041	0 (0)	*	*
Alcohol use										
Never	45 (45)	12 (11.76)	17 (25)	7 (7)	6 (5.88)			26 (38.24)		
<50 PW	2 (2)	18 (17.64)	6 (8.82)	9 (9)	8 (7.84)	5.866 (1.45–2.365)	0.00	1 (1.47)	2.949 (0.834–1.042)	0.093
50–100 PW	1 (1)	0 (0)	1 (1.47)	3 (3)	8 (7.84)	1.265 (7.264–0.220)	0.792	0 (0)	0.918 (9.216–0.092)	0.942
>100 PW	1 (1)	0 (0)	0 (0)	0 (0)	4 (3.92)	*	*	0 (0)	*	*
Chewing habit										
Never	40 (40)	16 (15.68)	12 (17.65)	16 (16)	11 (10.78)			3 (4.41)		
≤10 PD	5 (5)	11 (10.78)	10 (14.71)	1 (1)	12 (11.76)	7.872 (2.2–2.806)	0.00	17 (25)	1.3 (3.79–4.908)	0.00
>10 PD	4 (4)	3 (2.94)	9 (13.24)	9 (9)	2 (1.96)	1.999 (5.81–6.881)	0.272	7 (10.29)	10.012 (3.01–3.322)	0.00

PW per week, PD per day, OR odd ratio, CI confidence interval

* No statistics could be done due to absence of positively weighted cases as two of the weighed variables were having null value

^a Calculated by χ^2 analysis comparing genotype distributions among case and control subjects

Table 10 Analysis of combination genotypes as the risk factors for different oral pathologies

CYP1A1	GSTM1–			GSTT1–			GSTM1–T1–		
	OCs	NMPs	Control	OCs	NMPs	Control	OCs	NMPs	Control
m1m1	8	10	7	1	1	3	2	1	0
m1m2	31	13	12	4	0	1	1	1	1
m2m2	14	8	2	1	2	0	1	0	0
χ^2	0.108	0.312		0.17	0.141		0.39*		
<i>P</i>	<0.05	<0.05		NS	NS				

NMPs nonmalignant pathologies, NS non-significant

* No statistics possible

can be detoxified and excreted by type I metabolic enzymes such as GSTM1 and GSTT1. According to the literatures available the increase of activity of type II metabolic enzymes and/or decrease of activity of types I metabolic enzymes can increase the risk for cancer [27, 28]. In addition to polymorphisms in phase II enzymes, genetic polymorphisms in the phase I enzyme cytochrome *P450IA1* gene were correlated with an elevated risk of susceptibility to chemical carcinogenesis [29]. Negative correlations were also ascertained by some study [30].

GSTM1 is involved in the detoxification of tobacco-related carcinogens, such as epoxides and hydroxylated metabolites of benzo[*a*]pyrene. Approximately 45% of the white population lacks a functional allele. In other ethnic groups, this proportion may be much higher. We observed a strong effect of *GSTM1* deletion on OC risk, with those having *GSTM1* deletion exhibiting a significantly increased risk (OR: 4.06; CI: 7.54–1.75), but the same did not induce the susceptibility to NMPs. Tobacco significantly modified this susceptibility (Table 6). This is consistent with prior works [31, 32]. Deletion of *GSTT1* gene also increased the risk of OCs by 50% (OR: 1.5; CI: 5.47–0.41) and the NMPs by 10% (OR: 1.10; CI: 5.05–0.24). We did not observe a protective association of the *GSTT1* deletion with OC and NMP risks. Further stratification analysis of OC risk associated with *GSTM1* and *GSTT1* did not show any significant interaction between GST genotypes and the habits of alcohol drinking and betel quid chewing. The genotypes of m1m2 or m2m2 were associated with a significantly increased both OC and NMP risk compared with the m1m1 genotype among controls.

Combined analysis of *GSTM1* and *GSTT1* polymorphisms demonstrated a statistically significant impact on head and neck carcinogenesis [30, 33]. There are also evidences of negative correlations in combination analyses [34, 35]. However, no relation between polymorphisms of combined *GSTM1* and *GSTT1* null genotypes was found with nonmalignant oral lesions in the present study.

Few studies have also been carried out on Indian population earlier. Most of them were hospital-based

case–control studies. The earliest study [20] showed a pretty high association in the development of precancerous oral lesions. A study on South Indian population [11] found the significantly higher distribution of *CYP1A1* m2 (Ile/Val) genotypes and significantly higher deletion frequencies of *GSTM1* (49%) and *GSTT1* (18%) genes among OCs. Another study reported strong associations of the same polymorphisms among OCs, leukoplakia and sub-mucous fibrosis patients, but the degrees of associations were lower than the earlier studies [18]. The same study was carried out on the Bengali population of Eastern India taking OCs and Leukoplakias into account also showed significant associations in this regard [19, 34]. Our study apart from the NMPs also showed similar associations.

In summary, we found tobacco smoking, *CYP1A1*/m2m2 genotype; *GSTM1*, *GSTT1* deletion genotypes had associations with OCs in the studied population. Gene–environment interaction analysis proved that tobacco smoking had synergistic interactions with *CYP1A1*/m2m2 genotype and with *GSTM1*, *GSTT1* deletion genotypes. Of the studied genotypes, only the *GSTT1* null genotype might be of use in differentiating between (developing) OC and other pathologies.

Irrespective of several cautions in order to have a clear picture in this regard our study has some limitations. We have analysed the population only with 102 representing cases of OCs. However, for cancers with a low incidence such as oral ones, 102 cases should be a relatively good number. Another fact is that our study was hospital-based and could result in biased selection in place of random sampling. It has been suggested that studies with hospital controls can provide lower risk estimates, since diseases of controls could be associated with the polymorphisms under study, previous meta- and pooled analysis assessed that these polymorphisms on head and neck cancers found no differences for hospital-based studies in relation to population-based studies [35, 36].

In comparison to other body sites, the mouth permits noninvasive, repetitive examinations in longitudinal studies of tobacco-associated acute and chronic diseases. So, the

recognition of risk factors designed to identify people at risk of developing cancer is essential for good medical practice after proper clinical check-ups. The associations and the underlying mechanisms of tobacco smoking with the metabolic enzymes gene polymorphisms still need further study with large-scale (population-based) samples and modified designs.

Acknowledgements We are grateful to the Secretary, Ramakrishna Mission Seva Pratishthan, Kolkata for giving permission to carry out the study and to National Tea Research Foundation, India for partial financial support.

References

- Lin SC, Chen YJ, Kao SY, Hsu MT, Lin CH, Yang SC, et al. Chromosomal changes in betel-associated oral squamous cell carcinoma and their relationship to clinical parameters. *Oral Oncol.* 2002;38:266–73.
- Bhattacharjee A, Chakraborty A, Purkaystha P. Prevalence of head and neck cancers in the North East—an institutional study. *Indian J Otolaryngol Head Neck Surg.* 2006;58:15–9.
- Ferlay J, Bray F, Pisani P, Parkin DM. Cancer incidence, mortality and prevalence worldwide. IARC Cancer Base No. 5. IARC Press: Lyon, France; 2004.
- Kawajiri K, Fujii-Kuriyama Y. Cytochrome P450 and human cancer. *Jpn J Cancer Res.* 1991;82:1325–35.
- Seidegard J, Guthenberg C, Pero RW, Mannervik B. The trans stilbene oxide-active glutathione transferase in human mononuclear leukocytes is identical with the hepatic glutathione transferase. *Biochem J.* 1987;246:783–5.
- Bendjemana K, Abdennebi M, Gara S, Jamal A, Ghanem A, Touati S, et al. Genetic polymorphism of glutathione S-transferases and N-acetyl transferases and nasopharyngeal carcinoma: the Tunisia experience. *Bull Cancer.* 2006;93(3):297–302.
- Bogaards JJ, van Ommen B, van Bladeren PJ. Purification and characterization of eight glutathione S-transferase isoenzymes of hamster. Comparison of subunit composition of enzymes from liver, kidney, testis, pancreas and trachea. *Biochem J.* 1992;286:383–8.
- Ana H. Genetic polymorphisms and metabolism of endocrine disruptors in cancer susceptibility. *Cad Saúde Pública.* 2002;18(2):357–78.
- Lin HJ, Han CY, Bernstein DA, Hsiao W, Lin BK, Hardy S. Ethnic distribution of the glutathione transferase Mu1-1 (GSTM1) null genotype in 1473 individuals and application to bladder cancer susceptibility. *Carcinogenesis.* 1994;15:1077–81.
- Wiencke JK, Pemble S, Ketterer B, Kelsey KT. Gene deletion of glutathione S-transferase: correlation with induced genetic damage and potential role in endogenous mutagenesis. *Cancer Epidemiol Biomarkers Prev.* 1995;4:253–9.
- Sreelekha TT, Ramadas K, Pandey M, Thomas G, Nalinakumari KR, Pillai MR. Genetic polymorphisms of CYP1A1, GSTM1 and GSTT1 genes in Indian Oral Cancer. *Oral Oncol.* 2001;37:593–8.
- Axell T, Pindborg JJ, Smith CJ, van der Waal I. Oral white lesions with special reference to precancerous and tobacco-related lesions: conclusions of an international symposium held in Uppsala, Sweden, May 18–21. *J Oral Pathol Med.* 1996;25:49–54.
- Krutchkoff DJ, Eisenberg E. Lichenoid dysplasia: a distinct histopathologic entity. *Oral Surg Oral Med Oral Pathol.* 1985;30:308–15.
- Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acid Res.* 1988;18(3):1215.
- Sato M, Sato T, Izumo T, Amagasa T. Genetic polymorphism of drug-metabolising enzymes and susceptibility to oral cancer. *Carcinogenesis.* 1999;20(10):1927–31.
- Sikdar N, Mahmud SA, Paul RR, Roy B. Polymorphism in CYP1A1 and CYP2E1 genes and susceptibility to leukoplakia in Indian tobacco users. *Cancer Lett.* 2003;195:33–42.
- Nair Urmila J, Nair J, Mathew B, Barch H. Glutathione S-transferase M1 and T1 null genotypes as risk factors for oral leukoplakia in ethnic betel quid/tobacco chewers. *Carcinogenesis.* 1999;20(5):743–8.
- Anantharaman D, Chaubal Pranay M, Kannan S, Bhisey Rajani A, Mahimkar Manoj B. Susceptibility to oral cancer by genetic polymorphisms at CYP1A1, GSTM1 and GSTT1 loci among Indians: tobacco exposure as a risk modulator. *Carcinogenesis.* 2007;28(7):1455–62.
- Sikdar N, Paul Ranjan B, Bidyut R. Glutathione S-transferase M3(A/A) genotype as a risk factor for oral cancer and leukoplakia among Indian tobacco smokers. *Int J Cancer.* 2004;109:95–101.
- Frederica PP. Molecular epidemiology: insights into cancer susceptibility, risk assessment, and prevention. *J Natl Cancer Inst.* 1996;88:496–509.
- Merletti F, Boffetta P, Ciccone G, Mashber A, Terracini B. Role of tobacco and alcohol beverages in the etiology of head and neck lesions, cancer of the oral cavity/oropharynx in Torino, Italy. *Cancer Res.* 1989;49:4919–24.
- Ko YC, Huang YL, Lee CH, Chen MJ, Lin LM, Tsai CC. Betel quid chewing, cigarette smoking, and alcohol consumption related to oral cancer in Taiwan. *J Oral Pathol Med.* 1995;24:450–3.
- Lu CT, Yen YY, Ho CS, Ko YC, Tsai CC, Hsieh CC, et al. A case-control study of oral cancer in Changhua County, Taiwan. *J Oral Pathol Med.* 1996;25:245–8.
- Hung H-C, Chuang J, Chien YC, Chern HD, Chiang CP, Kuo YS, et al. Genetic polymorphisms of CYP2E1, GSTM1 and GSTT1; environmental factors and risk of oral cancer. *Cancer Epidemiol Biomarkers Prev.* 1997;6:901–5.
- Peters Edward S, McClean Michael D, Marsit Carmen J, Luckett B, Kelsey KT. Glutathione S-transferase polymorphisms and the synergy of alcohol and tobacco in oral, pharyngeal and laryngeal carcinoma. *Cancer Epidemiol Biomarkers Prev.* 2006;15(11):2196–202.
- Shao GZ, Hu Z, Li EM, Li J, Wen BG. Relationship between the GSTM1 genetic polymorphism and susceptibility to squamous cell carcinoma of esophagus. *Shantou Daxue Yixueyuan Xuebao.* 1999;12:1–3.
- Ketterer B, Harris JM, Talaska G, Meyer DJ, Pemble SE, Taylor JB, et al. The human glutathione S-transferase supergene family; its polymorphism and its effects on susceptibility to lung cancer. *Environ Health Perspect.* 1992;98:87–94.
- Kawajiri K, Nakachi K, Imai K, Yoshi A, Shinoda N, Watanabe J. Identification of genetically high risk individuals to lung cancer by DNA polymorphism of the cytochrome P450A1 gene. *FEBS Lett.* 1990;263:131–3.
- Oude Ophuis MB, van Lieshout Esther MM, Roelofs Hennie MJ, Peters Wilbert HM, Manni Johannes J. Glutathione S-transferase M1 and T1 and cytochrome P450A1 polymorphisms in relation to the risk for benign and malignant head and neck lesions. *Cancer.* 1998;5:936–43.
- Olshan AF, Weissler MC, Watson MA, Bell DA. GSTM1, GSTT1, GSTP1, CYP1A1 and NAT1 polymorphisms, tobacco use and the risk of head and neck cancer. *Cancer Epidemiol Biomarkers Prev.* 2000;9:185–91.
- Nazar-Stewart V, Vaughan Thomas L, Burt Richard D, Chen C, Berwick M, Swanson GM. Glutathione S-transferase M1 and

- susceptibility to nasopharyngeal carcinoma. *Cancer Epidemiol Biomarkers Prev.* 1999;8:547–51.
32. Varela-Lema L, Ruano-Ravina A, Juiz Crespo MA, Kelsey KT, Loidi L, Barros-Dios JM. CYP1A1, mEH and GSTM1 polymorphisms and risk of oral and pharyngeal cancer: a *Spanish* case-control study. *J Oncol.* 2008;741310:1–11.
33. Siraj Abdul K, Ibrahim M, Al-Rasheed M, Abubaker J, Bu R, Siddiqui Shakaib U, et al. Polymorphisms of selected xenobiotic genes contribute to the development of papillary thyroid cancer susceptibility in Middle Eastern population. *BMC Med Genet.* 2008;9:61.
34. Sikdar N, Datta S, Dey B, Paul RR, Panda KC, Ray B. Homozygous null genotype at glutathione S-transferase M1 locus as a risk factor for oral squamous cell carcinoma in Indian tobacco users. *Int J Hum Genet.* 2005;5(1):37–44.
35. Hashibe M, Brennan P, Strange RC, Bhisey R, Cascorbi I, Lazarus P, et al. Meta- and pooled analysis of GSTM1, GSTT1, GSTP1, and CYP1A1 genotypes and risk of head and neck cancer. *Cancer Epidemiol Biomarkers Prev.* 2003;12:1509–17.
36. Hung RJ, Boffetta P, Brockmoller J, Butkiewicz D, Cascorbi I, Clapper ML, et al. CYP1A1 and GSTM1 genetic polymorphisms and lung cancer risk in Caucasian non-smokers: a pooled analysis. *Carcinogenesis.* 2003;24(5):875–82.