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ORIGINAL ARTICLE

Metabolic Phase I (CYPs) and Phase II (GSTs) Gene Polymorphisms and Their Interaction with Environmental Factors in Nasopharyngeal Cancer from the Ethnic Population of Northeast India

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Abstract Multiple genetic and environmental factors and their interaction are believed to contribute in the pathogenesis of Nasopharyngeal Cancer (NPC). We investigate the role of Metabolic Phase I (CYPs) and Phase II (GSTs) gene polymorphisms, gene-gene and gene-environmental interaction in modulating the susceptibility to NPC in Northeast India. To determine the association of metabolic gene polymorphisms and environmental habits, 123 cases and 189 controls blood/swab samples were used for PCR and confirmed by Sanger sequencing. Analysis for GSTM1 and GSTT1 gene polymorphism was done by multiplex PCR. The T3801C in the 3′-flanking region of CYP1A1 gene was detected by PCR-RFLP method. The Logistic regression analysis was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI). The GSTM1 null genotype alone (OR = 2.76) was significantly associated with NPC risk ($P < 0.0001$). The combinations of GSTM1 null and GSTT1 null genotypes also higher, 3.77 fold ($P < 0.0001$), risk of NPC, while GSTM1 null genotype along with CYP1A1 T3801C TC + CC genotype had 3.22 ($P = 0.001$) fold risk. The most remarkable risk was seen among individual carrying GSTM1 null, GSTT1 null genotypes and CYP1A1 T3801C TC + CC genotypes (OR = 5.71, $P = 0.001$). Further; analyses demonstrate an enhanced risk of NPC in smoked meat (OR = 5.56, $P < 0.0001$) and fermented fish consumers (OR = 5.73, $P < 0.0001$) carrying GSTM1 null genotype. An elevated risk of NPC was noted in smokers (OR = 12.67, $P < 0.0001$) and chewers (OR = 5.68, $P < 0.0001$) with GSTM1 null genotype. However, smokers had the highest risk of NPC among individuals carrying GSTT1 null genotype (OR = 4.46, $P = 0.001$) or CYP1A1 T3801C TC + CC genotype (OR = 7.13, $P < 0.0001$). The association of null genotypes and mutations of metabolic neutralizing genes along with the environmental habits (tobacco smokers and chewers, smoke meat, fermented fishes) can be used as a possible biomarker for early detection and preventive measure of NPC.

Keywords Nasopharyngeal carcinoma (NPC) · Polymorphisms in metabolizing genes · Gene-gene interaction · Tobacco smokers and chewers · Gene-environment interaction · Northeast India

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Introduction

With an incidence well, under 1 per 100,000 populations per annum nasopharyngeal carcinoma (NPC) is rare in most parts around the world, but much more common among Cantonese Chinese, natives of South-East Asia, Arabs living in North Africa, Eskimos in Arctic and Native Americans [1]. It is an uncommon malignancy in Indian subcontinent except for the Northeastern states, especially Nagaland, Manipur, Mizoram and Sikkim [2]. These racial and geographic distributions of NPC suggest the involvement of both environmental and genetic factors for its development.

Major risk factors associated with NPC include Epstein-Barr virus infection [3, 4], consumption of salted fish [5], other preserved foods and condiments [6, 7], uses of herbal medicine [8, 9], cigarette smoking [10, 11] and/or alcohol

consumption [12, 13] and genetic susceptibility, e.g. mutations in one or more genes, family history of NPC, genetic polymorphism, etc. [14]. Polycyclic aromatic hydrocarbons (PAHs), *N*-nitrosamines, and heterocyclic aromatic amines are known components of such dietary items, including tobacco [15]. Ethanol and acetaldehyde are known carcinogens present in alcoholic drinks [16]. These compounds bind to DNA to form DNA adducts that may lead to initiation of carcinogenesis [17]. Phase I (CYPs) and Phase II (GSTs) enzymes activate and detoxify the carcinogens before eliminating from the body [18, 19]. However, inter-individual genetic variation may alter enzymatic activity and subsequently carcinogens, activation or deactivation, thereby increasing susceptibility to cancer risk [20]. Cytochrome P450 1A1 (CYP1A1), is a Phase I enzyme, included in the cytochrome P450 super family. One of its common polymorphism CYP1A1 T3801C (CYP1A1-*MspI* or CYP1A1*2A or rs4646903) has been associated with higher induction of CYP1A1. The higher enzyme activity would result in increased levels of carcinogenic intermediates, leading to greater risk of cancer development. The association of this CYP1A1 single nucleotide polymorphism (SNP) with cancer (e.g. head and neck, lung, breast cancer) was well documented [21–23]. Glutathione S-transferases (GSTs) are another group of enzymes involved in Phase II detoxification of carcinogens. Homozygous deletion genotype of its two common isoforms, GSTM1 and GSTT1 abolished enzyme activity thereby increasing susceptibilities to carcinogens and has been linked with cancers [24–27].

Complex disease such as cancer results from interactions of multiple genetic and environmental factors. Studying these factors singularly cannot explain the underlying pathogenic mechanism of the disease [28]. Extensive investigations have been carried in NPC with the genetic and environmental risk factors with less emphasis on their interaction. Here, we determined a high degree gene-gene and gene-environment interactions that modulate an individual's susceptibility to NPC. In this case-control study the effect of polymorphisms in major Metabolic Phase I (CYPs) CYP1A1, and Phase II (GSTs) GSTM1 and GSTT1 gene polymorphisms on NPC, and their differential effect according to diet (smoked meat and fermented fish) and lifestyle (tobacco-betel quid chewing and smoking) were investigated. Further, the degree of risk of NPC among individuals carrying more than one unfavourable genotype was also determined.

Materials and Methods

Ethical Statement

The study was approved by the Institutional Review Board (IRB), Assam University, Silchar and written consent was taken from all the participating subjects. All possible precautions were taken to avoid any cross contamination while collecting as well as processing the samples.

Sample Collection

A population-based case-control study of NPC was conducted among the ethnic population (viz., *Manipuri, Naga* and *Mizo*) of Northeastern States of India. The oral swab/peripheral blood of 123 histological confirmed NPC cases (diagnosed between 2012 and 2014) and 189 healthy controls (without family history of cancer) were collected. Controls were individually matched to cases in sex, age, ethnicity and neighbourhood.

All the subjects were interviewed using a standard questionnaire regarding tobacco habits (smoking and betel quid chewing), alcohol drinking and smoked meat and fermented fish intake. For dietary habits, subjects were divided to *never* (who do not consume), *regularly* (who consumed weekly or more) and *occasionally* (consuming monthly or biweekly). Smokers and chewers were defined as having smoked or chewed at least 1/day for six months. Those who had not smoked or chewed betel quid were defined as non smokers and chewers. They were further categorized based on their frequency of consumption. Heavy smokers were those who smoked at least ≥20 bidis/cigarettes per day for ≥20 years, and light smokers where those who smoked <20 bidis/cigarettes per day for <20 years. Whereas, heavy chewers where those who chewed ≥10 doses per day for ≥20 years, and those who chewed <10 doses per day for <20 years were defined as light chewers. Similarly, subjects who had drunk alcoholic beverages at least once a week for more than one year previously were defined as drinkers, and non-drinkers were those who had not drunk alcohol. *Alcohol drinking* was categorized as *light drinkers* (< 5 drinks per week for <20 years) and *heavy drinkers* (≥ 5 drinks per week for ≥20 years).

DNA Extraction and Genotyping

The genomic DNA was isolated from the collected blood samples were digested in lysis buffer and incubated overnight at 37 °C. The DNA was subsequently isolated by phenol/chloroform/ isoamylalcohol [29] method followed by ethanol precipitation and re-suspended in TE buffer and stored at −20 °C for further used. GSTT1 null and GSTM1 null genotypes were revealed by multiplex PCR-based assays with exon 7 of CYP1A1 gene as an internal control. The forward (F) and reverse (R) primers used for amplification were as follows: for GSTT1, F5′−TTCCTTACTGGTCCTCACATTCTC-3′ and R5′−TCACGGGATCATGGCCAGCA-3′; for GSTM1, F5′−GAACTCCCTGAAAAGCTAAAGC-3′ and R5′−GTTGGGCTCAAATATACGGTGG-3′; and for CYP1A1, F5′−GAACTGCCACTTCAGCTGTCT-3′ and

R5′–GCTGCATTTGGAAGTGCTC-3′ [30]. The PCR programme was performed at 95 °C for 5 min for the initial denaturation, following 30 cycles of denaturation at 95 °C for 30 s, annealing at 59 °C for 45 s, extension at 72 °C for 30 s and final extension at 72 °C for 5 mins. A 480, 315 and 215 bp amplicons represents the *GSTT1*, *CYP1A1* and *GSTM1* genes.

The polymorphisms ascribed to T3801C in the 3′-flanking region of *CYP1A1* gene was detected by PCR-RFLP method. PCR amplification of a 343-base DNA fragment containing a *MspI* restriction site was performed, using the primers 5′–TAGGAGTCTTGTCT CATGCCTT-3′and 5′–CAGTGAAGAGGTGTAGCCGC T-3′ [31]. The PCR programme was performed at 95 °C for 5 min for the initial denaturation, following 30 cycles of denaturation at 95 °C for 30 s, annealing at 62 °C for 45 s, extension at 72 °C for 30 s and final extension at 72 °C for 5 mins. For the CYP1A1 T3801C genotype analysis, a 343 bp fragment was digested by MspI restriction enzyme (New England BioLabs, USA); a single 343 bp fragment represents the wild-type allele (TT), three fragments of 343, 200 and 143 bp indicates for the heterozygous (TC) and two fragments of 200 and 143 bp for the variant allele (CC). The RFLP results were confirmed by sequencing 10% of the randomly selected samples from both cases and controls by Sanger sequencing using Genetic Analyzer 3500, Applied Biosystems (Molecular Medicine Lab, Department of Biotechnology, Assam University, Silchar, India).

Statistical Analysis

Statistically significant differences between cases and controls for demographic characteristics were assessed by χ^2 test. The association between the lifestyle habits (diet, smoking and tobacco-betel quid chewing) and genetic factors (GSTM1, GSTT1 and CYP1A1 T3801C genotypes) and NPC risk was analyzed by calculating odds ratios (ORs), 95% confidence intervals (95% CI), and their corresponding *P*-values. The gene-gene interaction and NPC risk, ORs were calculated for all the genotypes in combination. Hypotheses generated prior to the study were that deficient GSTM1, GSTTl and CYP1A1 T3801C genotypes are NPC risk factors. It was also believed that their impact may differ depending on lifestyle of the patients. The gene–environment interactions, stratified variables (genotype X environmental factor) were generated and included in the logistic model simultaneously. A *P*-value of less than 0.05 was considered to be statistically significant. Departures from Hardy-Weinberg equilibrium for CYP1A1 T3801C, genotype was evaluated by comparing the expected frequencies to observed genotype frequencies using χ^2 tests.

Results

Characteristics of the Study Population

The characteristics of the NPC patients and controls are represented in Table 1. There were no statistical differences between the cases and controls in terms of sex ($P = 0.7912$) and age ($P = 0.9203$). Significant variations were observed in consumption of smoked meat ($P < 0.0001$), fermented fish ($P = 0.0014$) and association with NPC risk. The ORs was (OR = 2.49, 95% CI: 1.33–4.67; $P = 0.004$) and (OR = 1.98; 95% CI: 1.09–3.6; $P = 0.024$). Smoking ($P < 0.0001$), tobacco-betel quid chewing ($P = 0.0059$); and alcohol drinking ($P = 0.0362$) showed a dose-dependent risk association with NPC (Fig. 1, Table 2). Regular smoked meat intake had 2.49 fold (95% CI: 1.33–4.67; $P = 0.004$) risk of NPC while fermented fish had nearly 2 fold (OR = 1.98; 95% CI: 1.09–3.6; $P = 0.024$) risk. However, heavy chewers and smoker had higher NPC risk; the ORs were 2.45 (95% CI: 1.24–4.7; $P = 0.009$) and 3.8 (95% CI: 1.95–7.7; P = <0.0001), respectively. No significant risk association was observed with alcohol drinking.

Polymorphism in Metabolic Genes and NPC Risk

The genotypes of GSTT1, GSTM1 and CYP1A1 T3801C were detected by the presence/absence of the desired band on 1.5% agarose gel (Fig. 2). The frequency distributions were 66.4% and 43.4% for GSTM1 null genotype, and 45.5% and 36.5%, for GSTT1 null genotype in cases and controls. The three genotypes of *CYP1A1* T3801C viz. TT, TC and CC had frequency distributions of 40.7%, 42.2%, 17.1% and 47.1%, 36.5%, 16.4% in cases and controls, respectively. Logistic regression method was used to analyse the association between GSTT1, GSTM1 and CYP1A1 T3801C genotypes, and NPC risk. It was found that GSTM1 null genotype was associated with 2.76 fold risk of NPC (95% CI: 1.61–4.71; $P < 0.0001$). GSTT1 and CYP1A1 T3801C genotypes did not show a significant risk to NPC in the study population (Table 2, Fig. 3).

Gene-Gene Interaction and NPC Risk

The interactions of GSTT1, GSTM1, CYP1A1 T3801C genotypes and risk of NPC were analyzed (Table 3). Significantly, elevated risk of NPC (OR = 3.77, 95% CI: 1.95–7.3; $P < 0.0001$) was observed among individuals carrying null genotypes of both GSTM1 and GSTT1. GSTM1 null genotypes in individual carrying CYP1A1 T3801C polymorphic variants had 3.2 fold increased risk of NPC (95% CI: 1.65–6.28; $P = 0.001$). However, highest risk of NPC (OR = 5.71, 95% CI: 2.11–15.45; $P = 0.001$) was observed in individuals carrying GSTM1 null, GSTT1 null and CYP1A1 T3801C polymorphic variants.

Table 1 Demographic characteristics and socioeconomic status of the study subjects

Variables	Case, n = 123 (%)	Control, n = 189 (%)	$\chi 2$	P value
Sex:			0.07	0.7913
Male	73 (59.3)	108 (57.2)		
Female	50 (40.7)	81 (42.8)		
Age Group:			0.01	0.9203
≤ 50	80 (65.1)	122 (64.6)		
> 50	43 (34.9)	67 (35.4)		
Ethnicity:			6.48	**0.0392**
Manipuri	29 (23.6)	54 (28.6)		
Naga	83 (67.5)	130 (68.8)		
Mizo	11 (8.9)	5 (2.6)		
Smoked meat intake			19.91	**<0.0001**
Never	28 (22.8)	78 (41.3)		
Occasionally	30 (24.4)	57 (30.2)		
Regularly	65 (52.8)	54 (28.5)		
Fermented fish intake			13.16	**0.0014**
Never	48 (39)	111 (58.7)		
Occasionally	20 (16.3)	28 (14.8)		
Regularly	55 (44.7)	50 (26.5)		
Tobacco-betel quid chewing			10.25	**0.0059**
Never	42 (34.2)	84 (44.5)		
Light	37 (30.1)	68 (36)		
Heavy	44 (35.7)	37 (19.5)		
Smoking			20.74	**<0.0001**
Never	63 (51.2)	143 (75.7)		
Light	22 (17.9)	21 (11.1)		
Heavy	38 (30.9)	25 (13.2)		
Alcohol intake			6.64	**0.0362**
Never	55 (44.7)	107 (56.6)		
Light	33 (26.8)	50 (26.5)		
Heavy	35 (28.5)	32 (16.9)		

Bold values indicate statistical significance ($P < 0.05$)

*Distribution in frequencies were tested by chi-square test, and $P < 0.05$ is considered statistically significant value

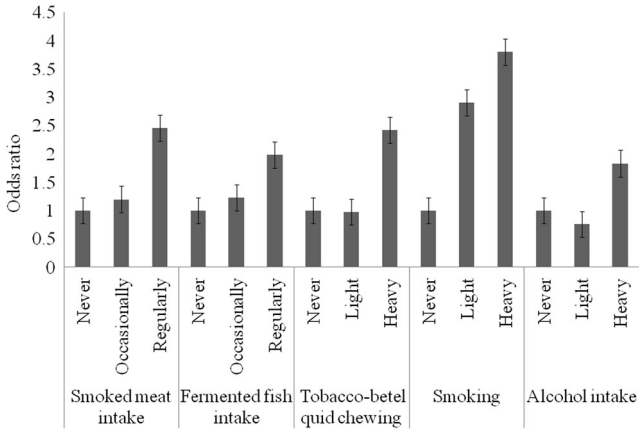

Fig. 1 Bar diagram showing the risk (Odds ratios) of NPC associated with environmental factors. Regular consumption of smoked meat (OR = 2.49) and fermented fish (OR = 1.98), heavy tobacco-betel quid chewing (OR = 2.42) and smoking (OR = 3.8) were associated with NPC risk. Alcohol drinking was not associated with NPC risk

Gene-Environment Interaction and NPC Risk

To investigate the potential gene–environment interaction analyses were carried out stratifying by lifestyle habits. The interactions between GSTM1 and environmental factors were examined (Table 4), a significant interaction was observed among the occasional and regular smoked meat consumers carrying GSTM1 null genotype. The ORs was 3.55 (95% CI, 1.50–8.41; P = 0.005) and 5.56 (95% CI, 2.91–10.62; $P < 0.0001$) in those carrying GSTM1 null genotypes compare to 0.82 (95% CI, 0.32–2.12; P = 0.81) and 2.47 (95% CI, 1.06–5.77; P = 0.053) among those with the gene present. Similarly, occasional and regular fermented fish consumers carrying GSTM1 null genotype had 6.23 fold (95% CI, 2.47–15.82; $P < 0.0001$) and 5.73 fold (95% CI, 2.66–12.34; $P < 0.0001$) elevated risk of NPC. Tobacco-betel quit chewers and smokers carrying GSTM1 null genotypes

Table 2 Distribution of *GSTM1*, *GSTT1* and *CYP1A1* T3801C genotype, smoked meat, fermented fish, tobacco and alcohol habits among the study subjects

Variables	Case, n = 123 (%)	Control, n = 189 (%)	ORs (95% CI)[a]	*P value
GSTM1				
Positive	41 (33.3)	107 (56.6)	1.0	Ref.
Negative	82 (66.4)	82 (43.4)	2.76 (1.61–4.716)	**<0.0001**
GSTT1				
Positive	67 (54.5)	120 (63.5)	1.0	Ref.
Negative	56 (45.5)	69 (36.5)	1.36 (0.8–2.31)	0.248
CYP1A1				
TT	50 (40.7)	89 (47.1)	1.0	Ref.
TC	52 (42.2)	69 (36.5)	1.44 (0.79–2.59)	0.225
CC	21 (17.1)	31 (16.4)	1.03 (0.48–2.21)	0.931
TC + CC	73 (59.3)	100 (52.9)	1.3 (0.75–2.23)	0.34
χ^2 (HWE), *P* value	1.34, 0.245	7.1, 0.007	–	–
Smoked meat intake				
Never	28 (22.8)	78 (41.3)	1.0	Ref.
Occasionally	30 (24.4)	57 (30.2)	1.2 (0.6–2.41)	0.601
Regularly	65 (52.8)	54 (28.5)	2.49 (1.33–4.67)	**0.004**
Fermented fish intake				
Never	48 (39)	111 (58.7)	1.0	Ref.
Occasionally	20 (16.3)	28 (14.8)	1.23 (0.57–2.67)	0.592
Regularly	55 (44.7)	50 (26.5)	1.98 (1.09–3.6)	**0.024**
Tobacco-betel quid chewing:				
Never	42 (34.2)	84 (44.5)	1.0	Ref.
Light	37 (30.1)	68 (36)	0.98 (0.52–1.84)	0.965
Heavy	44 (35.7)	37 (19.5)	2.42 (1.24–4.7)	**0.009**
Smoking				
Never	63 (51.2)	143 (75.7)	1.0	Ref.
Light	22 (17.9)	21 (11.1)	2.9 (1.3–6.49)	**0.009**
Heavy	38 (30.9)	25 (13.2)	3.8 (1.95–7.7)	**<0.0001**
Alcohol intake				
Never	55 (44.7)	107 (56.6)	1.0	Ref.
Light	33 (26.8)	50 (26.5)	0.76 (0.39–1.48)	0.433
Heavy	35 (28.5)	32 (16.9)	1.83 (0.92–3.65)	0.084

Bold values indicate statistical significance ($P < 0.05$)

Ca/Co Case/Control, *Ca* cases, *Co* controls

*Fisher's exact test used to calculate *P* value and $P < 0.05$ considered as statistically significance

[a] Odds adjusted for sex, age, ethnicity, smoked meat, fermented fish, smoking, tobacco-betel quid, alcohol and CYP1A1 T3801C, GSTM1 and GSTT1 genotypes as appropriate

showed a dose-dependent risk association of NPC. The ORs was 2.81 (95% CI, 1.29–6.12; $P = 0.012$) and 5.68 (95% CI, 2.46–13.08; $P < 0.0001$), respectively, in light and heavy chewers, carrying GSTM1 null genotype compared to 0.88 (95% CI, 0.37–2.13; $P = 0.825$) and 2.24 (95% CI, 0.94–5.35; $P = 0.111$), respectively, for those with the GSTM1 gene present. Light smokers with GSTM1 null individuals had 7.84 fold (95% CI, 2.80–21.99; $P < 0.0001$) increased risk of NPC. However, highest risk of NPC was observed in heavy smokers (OR = 12.67, 95% CI, 4.95–32.39; $P < 0.0001$) carrying GSTM1 null genotypes.

For GSTT1 genotype, a statistically significant interaction was observed among regular smoked meat and fermented fish consumers. The ORs was 3.99 (95% CI, 1.84–8.68; $P = 0.001$) and 3.50 (95% CI, 1.73–7.09; $P = 0.001$), respectively, for individuals with GSTT1 null compare to 3.46 (95% CI, 1.65–7.23; $P = 0.001$) and 2.27 (95% CI, 1.15–4.49; $P = 0.002$), respectively, for those with the *GSTT1* gene present (Table 5). However, no interactions between GSTT1 and occasional smoked meat and fermented fish consumers were noticed. Heavy tobacco-betel quid chewers, carrying GSTT1 null genotype had 3.51 fold (95% CI, 1.44–9.42) elevated risk

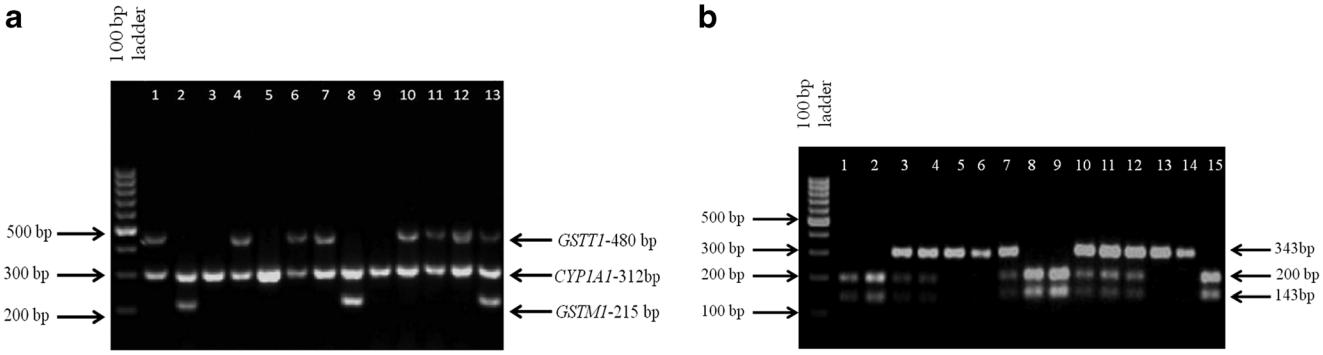

Fig. 2 Polymorphisms in CYP1A1 T3801C, GSTM1 and GSTT1 metabolic genes **a** GSTT1 and GSTM1 polymorphism: Ethidium bromide stained gel *GSTM1* null genotype (lanes 1, 3, 4, 5, 6, 7, 9, 10, 11 and 12); GSTT1 null genotype (lanes 2, 3, 5, 8 and 9); GSTM1-GSTT1 wild type genotype (lane 13) and both GSTM1-GSTT1 null genotypes (lanes 3, 5 and 9); **b** CYP1A1 T3801C polymorphism: Ethidium bromide stained gel CYP1A1 TT wild genotype (lanes 5, 6, 13 and 14); CYP1A1 TC heterozygous genotype (lanes 3, 4, 7, 10, 11, and 12); CYP1A1 CC mutant genotype (lanes 1, 2, 8, 9, and 15)

of NPC while there was no-risk association in light chewers. Similarly, significant interaction was also observed in smokers carrying GSTT1 null genotype. The OR was 4.60 (95% CI, 1.29–16.4; $P = 0.025$) in light and 4.46 (95% CI, 1.89–10.56; $P = 0.001$) in heavy smokers compare to an OR of 2.89 (95% CI, 1.31–6.37; $P = 0.01$) and 4.82 (95% CI, 2.21–10.54; $P < 0.0001$) for those with the *GSTT1* gene present.

We also observed a significant interaction of CYP1A1 polymorphisms with lifestyle habits (Table 6). Regular consumption of smoked meat in individuals with CYP1A1 T3801C variant (TC + CC) genotypes had 4.12 fold (95% CI, 1.89–8.99; $P < 0.0001$) increased risk of NPC whereas wild-type carriers had 4.38 fold (95% CI, 1.85–10.35; $P = 0.001$) risk. Similarly, regular fermented fish consumers carrying the CYP1A1 T3801C variant genotypes had an OR of 3.32 (95% CI, 1.56–7.05; $P = 0.003$) versus 2.53 (95% CI, 1.20–5.35; $P = 0.023$) in individuals with the TT genotype. A significant interaction was noted among heavy tobacco-betel quid chewers and smokers in those individual polymorphic for CYP1A1 T3801C. The ORs was 2.86 (95% CI, 1.20–6.82; $P = 0.03$) and 7.13 (95% CI, 2.88–17.68; $P < 0.0001$), respectively, for individuals carrying CYP1A1 T3801C variant genotypes, which was significantly higher than individuals with CYP1A1 T3801C TT genotype .

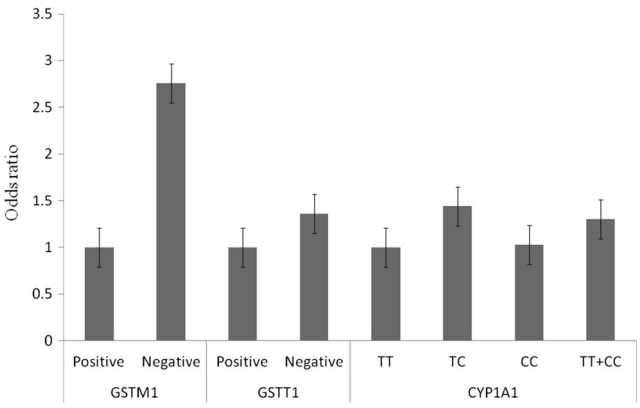

Fig. 3 Bar diagram showing the risk (Odds ratios) of NPC associated with CYP1A1 T3801C, GSTT1 and GSTM1 polymorphism. GSTM1 null genotypes (OR = 2.49) was associated with NPC risk. CYP1A1 T3801C and GSTT1 polymorphisms were not associated with NPC risk

Discussion

To the best of our knowledge, this is the first report on the impact of combined effects of *CYP1A1* (T3801C), *GSTT1* and *GSTM1* genes with the tobacco habits, smoked meat and fermented fish consumption in the susceptibility to NPC in the ethnic northeast Indian population.

We found that individuals with tobacco habits were at an increased risk of NPC. Smokers were significantly associated with the risk of NPC development. These results are compatible with previous epidemiological data that show a strong correlation between cancer and smoking [17, 32]. The habits of smoking (cigarette/bidi), tobacco-betel quid (with or without tobacco) chewing and other tobacco products like *gutkha*, *paan-masala*, *khaini* are endemic throughout the Indian subcontinent and shown the relation between chewing and head and neck cancer [24, 33]. Our data showed significant association between smoked meat and fermented fish consumption; and NPC risk. These are traditional staple foods consumed in several regions of Northeast India, especially NPC-endemic areas. Smoked meat consumption is linked with high prevalence of NPC [9], and in our study, also observed a 2.4 fold increased risk to NPC.

CYP1A1, GSTT1 and GSTM1 belong to a super family of Phase I and Phase II xenobiotics metabolizing enzymes. These enzymes play a vital role in resisting a large variety of chemical carcinogens and environmental toxicants that are probably associated with cancer risk [34, 35]. Many studies showed conflicting role of GSTT1 null and GSTM1 null genotypes on NPC risk [36, 37]. Here, we found a significant association between GSTM1 (2.76 fold) null genotype and

Table 3 Odds ratios for the interaction of *GSTM1*, *GSTT1* and *CYP1A1* T3801C genotypes in the study subjects

Genotypes	Cases, n = 123(%)	Controls, n = 189 (%)	ORs (95% CI)	*P value
GSTM1and GSTT1				
M1 (+/+) and T1 (+/+)	26 (21.2)	67 (35.4)	1.0	Ref.
M1 (+/+) and T1 (−/−)	15 (12.2)	40 (21.2)	0.96 (0.45–2.03)	0.928
M1 (−/−) and T1 (+/+)	41 (33.3)	53 (28.1)	1.95 (1.06–3.59)	**0.031**
M1 (−/−) and T1 (−/−)	41 (33.3)	29 (15.3)	3.77 (1.95–7.3)	**<0.0001**
*GSTM1*and *CYP1A1* T3801C				
M1 (+/+) and TT	18 (14.6)	51 (27)	1.0	Ref.
M1 (+/+) and TC or CC	23 (18.7)	56 (29.6)	1.16 (0.57–2.39)	0.716
M1 (−/−) and TT	32 (26)	38 (20.1)	2.39 (1.17–4.85)	**0.021**
M1 (−/−) and TC or CC	50 (40.7)	44 (23.3)	3.22 (1.65–6.28)	**0.001**
*GSTT1*and *CYP1A1* T3801C				
T1 (+/+) and TT	24 (19.5)	53 (28.1)	1.0	Ref.
T1 (+/+) and TC or CC	43 (34.9)	67 (35.4)	1.46 (0.78–2.7)	0.229
T1 (−/−) and TT	26 (21.2)	36 (19.1)	1.94 (0.97–3.87)	0.057
T1 (−/−) and TC or CC	30 (24.4)	33 (17.4)	1.52 (0.77–3.11)	0.216
GSTM1, *GSTT1* and *CYP1A1* T3801C				
M1 (+/+), T1 (+/+) and TT	9 (7.3)	30 (15.9)	1.0	Ref.
M1 (+/+), T1 (+/+) and TC or CC	17 (13.8)	37 (19.6)	1.53 (0.59–3.92)	0.374
M1 (−/−), T1 (+/+) and TT	15 (12.2)	23 (12.2)	2.17 (0.8–5.84)	0.124
M1 (−/−), T1 (+/+) and TC or CC	26 (21.2)	30 (15.9)	2.88 (1.61–7.18)	**0.023**
M1 (+/+), T1 (−/−) and TT	9 (7.3)	21 (11.1)	1.42 (0.48–4.2)	0.517
M1 (+/+), T1 (−/−) and TC or CC	6 (4.9)	19 (10)	1.05 (0.32–3.43)	0.932
M1 (−/−), T1 (−/−) and TT	17 (13.8)	15 (7.9)	3.77 (1.36–10.45)	**0.011**
M1 (−/−), T1 (−/−) and TC or CC	24 (19.5)	14 (7.4)	5.71 (2.11–15.45)	**0.001**

Bold values indicate statistical significance ($P < 0.05$)

Ca/Co Case/Control, *Ca* cases, *Co* controls

*Fisher's exact test used to calculate *P* value and $P < 0.05$ considered as statistically significance

incidence of NPC, but not with *GSTT1* gene. However, recent meta-analysis demonstrate higher incidence of NPC in individuals carrying the defective *GSTT1* and *GSTM1* genes [38]. As reported earlier, CYP1A1 T3801C polymorphism was not associated with NPC risk [39] and other cancers [40].

NPC is polygenic disease and polymorphism in individual genes cannot explain the underlying pathogenic mechanism. To understand such complex diseases the cumulative effect of many polymorphisms is more likely important. To date, no studies have examined the risk conferred by the combination of CYP1A1, GSTT1 and GSTM1 polymorphisms in the endemic part of northeast Indian population. Studies in an endemic region have shown the elevated risk of NPC in both GSTT1 null and GSTM1 null genotypes [36, 37]. Similarly, we found that GSTM1 null genotype in the absence of GSTT1 genotype had a 3.77 fold increased risk of NPC. Significant interaction was also observed between GSTMI and CYP1A1 genes ($P = 0.001$). However, highest risk of NPC (5.71 folds) was observed in individual carrying the defective genotypes of GSTT1, GSTM1 and CYP1A1 T3801C, suggesting that cross talk between these genes might modulate susceptibility towards NPC. Similar results were reported in head and neck cancers (HNC) [32, 41].

Furthermore, significant gene-environment interactions that further modify the risk of NPC were noted. When a combine effect of diet (smoked meat and fermented fish) and genotypes were considered, highest joint effect was observed in individual with GSTM1 null or GSTT1 null genotypes ($P < 0.0001$). Significant interaction was also observed with CYP1A1 T3801C polymorphic variants, which modulate the risk of NPC ($P = 0.001$). These foods are highly contaminated by nitrosamines and nitrosamine precursors as a result of processing. Smoked meat contains nitrosodimethylamine (NDMA), nitrosodiethylamine (NDEA) and

Table 4 Association between *GSTM1* genotype and NPC, stratified by smoked meat, fermented fish, smoking and tobacco-betel quid habits

Variables	GSTM1 positive			GSTM1 negative		
	Ca/Co	OR (95% CI)	*P value	Ca/Co	OR (95% CI)	*P value
Smoked meat intake						
Never	13/44	1.0	Ref.	15/34	1.49 (0.63–3.52)	0.386
Occasionally	9/37	0.82 (0.32–2.12)	0.810	21/20	3.55 (1.50–8.41)	**0.005**
Regular	19/26	2.47 (1.06–5.77)	0.053	46/28	5.56 (2.91–10.62)	**<0.0001**
Fermented fish intake						
Never	17/59	1.0	Ref.	31/52	2.07 (1.03–4.14)	0.057
Occasionally	2/18	0.39 (0.08–1.77)	0.345	18/19	6.23 (2.47–15.82)	**<0.0001**
Regular	22/30	2.55 (1.19–5.47)	**0.020**	33/20	5.73 (2.66–12.34)	**<0.0001**
Smoking						
Never	18/76	1.0	Ref.	45/67	2.84 (1.5–5.35)	**0.001**
Light	9/14	2.71 (1.03–7.13)	0.054	13/7	7.84 (2.80–21.99)	**<0.0001**
Heavy	14/17	3.48 (1.47–8.24)	**0.008**	24/8	12.67 (4.95–32.39)	**<0.0001**
Tobacco-betel quid chewing						
Never	15/17	1.0	Ref.	27/37	2.29 (1.07–4.88)	**0.039**
Light	11/39	0.88 (0.37–2.13)	0.825	26/29	2.81 (1.29–6.12)	**0.012**
Heavy	15/21	2.24 (0.94–5.35)	0.111	29/16	5.68 (2.46–13.08)	**<0.0001**

Bold values indicate statistical significance ($P < 0.05$)

Ca/Co Case/Control, *Ca* cases, *Co* controls

*Fisher's exact test used to calculate *P* value and $P < 0.05$ considered as statistically significance

Table 5 Association between *GSTT1* genotype and NPC, stratified by by smoked meat, fermented fish, smoking and tobacco-betel quid habits

Variables	GSTT1 positive			GSTT1 negative		
	Ca/Co	OR (95% CI)	*P value	Ca/Co	OR (95% CI)	*P value
Smoked meat intake						
Never	16/49	1.0	Ref.	12/29	1.27 (0.53–3.02)	0.654
Occasionally	14/40	1.22 (0.55–2.73)	0.682	14/17	2.52 (1.03–6.16)	0.059
Regular	35/31	3.46 (1.65–7.23)	**0.001**	30/23	3.99 (1.84–8.68)	**0.001**
Fermented fish intake						
Never	28/71	1.0	Ref.	20/40	1.27 (0.64–2.52)	0.593
Occasionally	13/20	1.65 (0.73–3.72)	0.279	7/8	2.22 (0.76–6.48)	0.227
Regular	26/29	2.27 (1.15–4.49)	**0.022**	29/21	3.50 (1.73–7.09)	**0.001**
Smoking						
Never	29/89	1.0	Ref.	34/54	1.93 (1.06–3.51)	**0.033**
Light	16/17	2.89 (1.31–6.37)	**0.010**	6/4	4.60 (1.29–16.4)	**0.025**
Heavy	22/14	4.82 (2.21–10.54)	**<0.0001**	16/11	4.46 (1.89–10.56)	**0.001**
Tobacco-betel quid chewing						
Never	24/55	1.0	Ref.	18/29	1.42 (0.62–3.02)	0.435
Light	22/43	1.17 (0.58–2.36)	0.721	15/25	1.38 (0.62–3.03)	0.536
Heavy	21/22	2.19 (1.02–4.67)	0.051	23/15	3.51 (1.58–7.82)	**0.003**

Bold values indicate statistical significance ($P < 0.05$)

Ca/Co Case/Control, *Ca* cases, *Co* controls

*Fisher's exact test used to calculate *P* value and $P < 0.05$ considered as statistically significance

Table 6 Association between *CYP1A1* T3801C genotype and NPC, stratified by smoked meat, fermented fish, smoking and tobacco-betel quid habits

Variables	*CYP1A1* TT			*CYP1A1* TC + CC		
	Ca/Co	OR (95% CI)	*P value	Ca/Co	OR (95% CI)	*P value
Smoked meat intake						
Never	12/42	1.0	Ref.	16/36	1.56 (0.66–3.69)	0.381
Occasionally	13/27	1.69 (0.68–4.19)	0.346	17/30	1.98 (0.83–4.72)	0.131
Regular	25/20	4.38 (1.85–10.35)	**0.001**	40/34	4.12 (1.89–8.99)	**<0.0001**
Fermented fish intake						
Never	19/50	1.0	Ref.	29/61	1.25 (0.63–2.48)	0.602
Occasionally	5/12	1.10 (0.35–3.43)	1.00	15/16	2.47 (1.03–5.88)	0.067
Regular	26/27	2.53 (1.20–5.35)	**0.023**	29/23	3.32 (1.56–7.05)	**0.003**
Smoking						
Never	27/67	1.0	Ref.	36/76	1.18 (0.65–2.13)	0.650
Light	8/5	3.97 (1.24–12.69)	**0.027**	14/16	2.17 (0.94–5.0)	0.078
Heavy	15/17	2.19 (0.97–4.95)	0.082	23/8	7.13 (2.88–17.68)	**<0.0001**
Tobacco-betel quid chewing						
Never	13/31	1.0	Ref.	29/53	1.3 (0.6–2.85)	0.557
Light	17/41	0.99 (0.42–2.32)	1.00	20/27	1.77 (0.75–4.17)	0.275
Heavy	20/17	2.81 (1.14–6.93)	**0.040**	24/20	2.86 (1.20–6.82)	**0.030**

Bold values indicate statistical significance ($P < 0.05$)

Ca/Co Case/Control, *Ca* cases, *Co* controls

*Fisher's exact test used to calculate *P* value and $P < 0.05$ considered as statistically significance

nitrosopyrrolidine (NPYR) which are known mutagen and has proven to be risk factors for NPC [35, 42]. Therefore, such an interaction is biologically possible as individual with the defective genotypes do not have proper enzyme activity and are more susceptible to carcinogens present in the preserved foods. To our knowledge, we reported for the first time a strong effect modification by diet of the association between metabolic genes and NPC.

In addition, we observed a significant interaction of metabolic gene with tobacco habits. Recent studies conducted in India, showed that GSTM1, GSTT1 and CYP1A1 genes are associated with cancer among chewers [41, 43]. Tobacco-betel quid chewing results in the exposure to tobacco specific nitrosamines (TSNA) and nitrosamines derived from areca or betel nut alkaloids, which are known carcinogens. In our study, chewers carrying the defective GSTM1 gene had 5.86 fold increased risk of NPC. Significance interaction was observed in chewers with GSTTI null or CYP1A1 T3801C polymorphic variants ($P < 0.05$). However, highest risk (12.67 fold) of NPC was observed in GSTM1 null individual with habits of smoking. Tobacco smoke contains over 60 potent carcinogens including polycyclic aromatic hydrocarbons, aromatic amines, *N*-nitroso compounds. These chemicals can generate reactive oxygen species (ROS), form bulky adducts; induce a variety of oxidative damage and single strand break [44, 45]. Similarly, smokers with defective GSTT1 gene (4.46 fold) were associated with NPC risk. A recent study conducted on head and neck cancer has also reported significant interactions of GSTT1 and GSTM1 gene polymorphisms with smoking [33]. NPC is strongly associated with smoking, and no study has been conducted that explore the role of CYP1A1 polymorphism in the risk of developing NPC in smokers. Here, we observed a significant increased (7.13 fold) risk of NPC in smokers carrying the CYP1A1 TC + CC genotypes. Our result is supported by previous studies conducted on HNC in northern and southern India [43, 46]. However, few studies [47, 48] did not find a relationship between smoking and risk of cancer with the CYP1A1 polymorphisms.

Besides the role of genetic and environmental factors, studies have also suggested the involvement of epigenetic changes towards cancer progression [49, 50]. Promoter methylation in tumor suppressor genes (TSGs) is thought to be a key event in the initiation and progression of cancer, including NPC [51–54]. Moreover, environmental factors such as tobacco, alcohol, diet and viral infection may also lead to a wide range of epigenetic changes that promote genomic instability and contribute to tumor development [55–57]. EBV load were found to be correlated with aberrant promoter hypermethylation of DAP-kinase, p16, RASSF1A and *TSLC1* genes in NPC [58, 59]. The viral genes are thought to have contributed

in epigenetic silencing either through activation of DNA methyltransferases (DNMT) [60] or interaction with transcriptional repression [61]. Other factors like diet and lifestyle have also showed higher frequency of E-cadherin, p15, p16INKa, MGMT, p14ARF, DAPK, GSTP1 and BRCA1 genes promoter methylation in head and neck cancers (HNC) [57, 62–67]. Carcinogens including nitrosamines, acetaldehyde, polyaromatic hydrocarbons (PAHs) like NNK, Benzo[a]pyrene etc., are common constituent of such factors which can impact methylation patterns by altering DNMT activity and leading to cancer development [68, 69].

Our findings confirm the role of environmental factors along with genetic polymorphisms as risks enhancers in the etiology of NPC among the ethnic population of northeast India.. However, the potential relationship between carcinogenic exposure and epigenetic changes cannot be neglected and are vital for understanding cancer development.

Conclusion

We showed that the null genotype of GSTM1 is a strong predisposing risk factor for NPC. The combine effects of variant genotypes (gene-gene interactions) indicate the risk for developing NPC. Further, the interaction between the GSTM1 null and GSTT1 null genotypes or CYPA1A T3801C polymorphic variant, and the environmental factors significantly modify the risk of NPC in the study population.

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Compliance with Ethical Standards

Conflict of Interest The authors declare no conflict of interest.

References

1. Chang ET, Adami HO (2006) The enigmatic epidemiology of nasopharyngeal carcinoma. Cancer Epidemiol Biomark Prev 15(10): 1765–1777. https://doi.org/10.1158/1055-9965.EPI-06-0353
2. Kataki AC, Simons MJ, Das AK, Sharma K, Mehra NK (2011) Nasopharyngeal carcinoma in the Northeastern states of India. Chin J Cancer 30(2):106–113
3. Ghosh SK, Singh AS, Mondal R, Kapfo W, Khamo V, Singh YI (2014) Dysfunction of mitochondria due to environmental carcinogens in nasopharyngeal carcinoma in the ethnic group of northeast Indian population. Tumour Biol J Int Soc Oncodev Biol Med. https://doi.org/10.1007/s13277-014-1897-x
4. Tsao SW, Yip YL, Tsang CM, Pang PS, Lau VM, Zhang G, Lo KW (2014) Etiological factors of nasopharyngeal carcinoma. Oral Oncol 50(5):330–338. https://doi.org/10.1016/j.oraloncology.2014.02.006
5. Jia WH, Luo XY, Feng BJ, Ruan HL, Bei JX, Liu WS, Qin HD, Feng QS, Chen LZ, Yao SY, Zeng YX (2010) Traditional Cantonese diet and nasopharyngeal carcinoma risk: a large-scale case-control study in Guangdong, China. BMC Cancer 10:446. https://doi.org/10.1186/1471-2407-10-446
6. Belbaraka R, Lalya I, Boulaamane L, Tazi M, Benjaafar N, Errihani H (2013) Dietary risk factors of undifferenced nasopharyngeal carcinoma: a case-control study. Tunis Med 91(6):406–409
7. Gallicchio L, Matanoski G, Tao XG, Chen L, Lam TK, Boyd K, Robinson KA, Balick L, Mickelson S, Caulfield LE, Herman JG, Guallar E, Alberg AJ (2006) Adulthood consumption of preserved and nonpreserved vegetables and the risk of nasopharyngeal carcinoma: a systematic review. Int J Cancer 119(5):1125–1135. https://doi.org/10.1002/ijc.21946
8. Hildesheim A, West S, DeVeyra E, De Guzman MF, Jurado A, Jones C, Imai J, Hinuma Y (1992) Herbal medicine use, Epstein-Barr virus, and risk of nasopharyngeal carcinoma. Cancer Res 52(11):3048–3051
9. Chelleng PK, Narain K, Das HK, Chetia M, Mahanta J (2000) Risk factors for cancer nasopharynx: a case-control study from Nagaland, India. Natl Med J India 13(1):6–8
10. Xue WQ, Qin HD, Ruan HL, Shugart YY, Jia WH (2013) Quantitative association of tobacco smoking with the risk of nasopharyngeal carcinoma: a comprehensive meta-analysis of studies conducted between 1979 and 2011. Am J Epidemiol 178(3):325–338. https://doi.org/10.1093/aje/kws479
11. Fachiroh J, Sangrajrang S, Johansson M, Renard H, Gaborieau V, Chabrier A, Chindavijak S, Brennan P, McKay JD (2012) Tobacco consumption and genetic susceptibility to nasopharyngeal carcinoma (NPC) in Thailand. Cancer Causes Control: CCC 23(12):1995–2002. https://doi.org/10.1007/s10552-012-0077-9
12. Ruan HL, Xu FH, Liu WS, Feng QS, Chen LZ, Zeng YX, Jia WH (2010) Alcohol and tea consumption in relation to the risk of nasopharyngeal carcinoma in Guangdong, China. Front Med China 4(4):448–456. https://doi.org/10.1007/s11684-010-0280-6
13. Chen L, Gallicchio L, Boyd-Lindsley K, Tao XG, Robinson KA, Lam TK, Herman JG, Caulfield LE, Guallar E, Alberg AJ (2009) Alcohol consumption and the risk of nasopharyngeal carcinoma: a systematic review. Nutr Cancer 61(1):1–15. https://doi.org/10.1080/01635580802372633
14. Bei JX, Li Y, Jia WH, Feng BJ, Zhou G, Chen LZ, Feng QS, Low HQ, Zhang H, He F, Tai ES, Kang T, Liu ET, Liu J, Zeng YX (2010) A genome-wide association study of nasopharyngeal carcinoma identifies three new susceptibility loci. Nat Genet 42(7):599–603. https://doi.org/10.1038/ng.601
15. Diggs DL, Huderson AC, Harris KL, Myers JN, Banks LD, Rekhadevi PV, Niaz MS, Ramesh A (2011) Polycyclic aromatic hydrocarbons and digestive tract cancers: a perspective. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 29(4):324–357. https://doi.org/10.1080/10590501.2011.629974
16. Lachenmeier DW, Przybylski MC, Rehm J (2012) Comparative risk assessment of carcinogens in alcoholic beverages using the margin of exposure approach. Int J Cancer 131(6):E995–1003. https://doi.org/10.1002/ijc.27553
17. Mondal R, Ghosh SK (2013) Accumulation of mutations over the complete mitochondrial genome in tobacco-related oral cancer from northeast India. Mitochondrial DNA 24(4):432–439. https://doi.org/10.3109/19401736.2012.760551
18. Xue W, Warshawsky D (2005) Metabolic activation of polycyclic and heterocyclic aromatic hydrocarbons and DNA damage: a review. Toxicol Appl Pharmacol 206(1):73–93. https://doi.org/10.1016/j.taap.2004.11.006
19. Shukla D, Dinesh Kale A, Hallikerimath S, Yerramalla V, Subbiah V, Mishra S (2013) Association between GSTM1 and CYP1A1

polymorphisms and survival in oral cancer patients. Biomed Pap Med Fac Univ Palacky Olomouc Czechoslovakia 157(4):304–310. https://doi.org/10.5507/bp.2013.028

20. Rodriguez-Antona C, Ingelman-Sundberg M (2006) Cytochrome P450 pharmacogenetics and cancer. Oncogene 25(11):1679–1691. https://doi.org/10.1038/sj.onc.1209377
21. Mota P, Moura DS, Vale MG, Coimbra H, Carvalho L, Regateiro F (2010) CYP1A1 m1 and m2 polymorphisms: genetic susceptibility to lung cancer. Rev Port Pneumol 16(1):89–98
22. Sabitha K, Reddy MV, Jamil K (2010) Smoking related risk involved in individuals carrying genetic variants of CYP1A1 gene in head and neck cancer. Cancer Epidemiol 34(5):587–592. https://doi.org/10.1016/j.canep.2010.05.002
23. Sergentanis TN, Economopoulos KP (2010) Four polymorphisms in cytochrome P450 1A1 (CYP1A1) gene and breast cancer risk: a meta-analysis. Breast Cancer Res Treat 122(2):459–469. https://doi.org/10.1007/s10549-009-0694-5
24. Mondal R, Ghosh SK, Choudhury JH, Seram A, Sinha K, Hussain M, Laskar RS, Rabha B, Dey P, Ganguli S, Nathchoudhury M, Talukdar FR, Chaudhuri B, Dhar B (2013) Mitochondrial DNA copy number and risk of oral cancer: a report from Northeast India. PLoS One 8(3):e57771. https://doi.org/10.1371/journal.pone.0057771
25. Nosheen M, Ishrat M, Malik FA, Baig RM, Kayani MA (2010) Association of GSTM1 and GSTT1 gene deletions with risk of head and neck cancer in Pakistan: a case control study. Asian Pac J Cancer Prev 11(4):881–885
26. Sharma A, Das BC, Sehgal A, Mehrotra R, Kar P, Sardana S, Phukan R, Mahanta J, Purkayastha J, Saxena S, Kapur S, Chatterjee I, Sharma JK (2013) GSTM1 and GSTT1 polymorphism and susceptibility to esophageal cancer in high- and low-risk regions of India. Tumour Biol J Int Soc Oncodev Biol Med 34(5):3249–3257. https://doi.org/10.1007/s13277-013-0897-6
27. Shukla RK, Tilak AR, Kumar C, Kant S, Kumar A, Mittal B, Bhattacharya S (2013) Associations of CYP1A1, GSTM1 and GSTT1 polymorphisms with lung cancer susceptibility in a Northern Indian population. Asian Pac J Cancer Prev 14(5):3345–3349
28. Ihsan R, Chauhan PS, Mishra AK, Yadav DS, Kaushal M, Sharma JD, Zomawia E, Verma Y, Kapur S, Saxena S (2011) Multiple analytical approaches reveal distinct gene-environment interactions in smokers and non smokers in lung cancer. PLoS One 6(12):e29431. https://doi.org/10.1371/journal.pone.0029431
29. Ghosh SK, Mondal R (2012) Quick diagnosis of female genital tuberculosis using multiplex fast polymerase chain reaction in Southern Assam, India. Int J Gynaecol Obstet Off Organ Int Fed Gynaecol Obstet 118(1):72–73. https://doi.org/10.1016/j.ijgo.2012.02.006
30. Mondal R, Ghosh SK, Talukdar FR, Laskar RS (2013) Association of mitochondrial D-loop mutations with GSTM1 and GSTT1 polymorphisms in oral carcinoma: a case control study from northeast India. Oral Oncol 49(4):345–353. https://doi.org/10.1016/j.oraloncology.2012.11.003
31. Kiruthiga PV, Kannan MR, Saraswathi C, Pandian SK, Devi KP (2011) CYP1A1 gene polymorphisms: lack of association with breast cancer susceptibility in the southern region (Madurai) of India. Asian Pac J Cancer Prev 12(8):2133–2138
32. Choudhury JH, Choudhury B, Kundu S, Ghosh SK (2014) Combined effect of tobacco and DNA repair genes polymorphisms of XRCC1 and XRCC2 influence high risk of head and neck squamous cell carcinoma in northeast Indian population. Med Oncol 31(8):67. https://doi.org/10.1007/s12032-014-0067-8
33. Choudhury JH, Ghosh SK (2014) Gene-environment interaction and susceptibility in head and neck cancer patients and in their first-degree relatives: a study of Northeast Indian population. J Oral Pathol Med Off Publ Int Assoc Oral Pathol Am Acad Oral Pathol. https://doi.org/10.1111/jop.12249
34. McIlwain CC, Townsend DM, Tew KD (2006) Glutathione S-transferase polymorphisms: cancer incidence and therapy. Oncogene 25(11):1639–1648. https://doi.org/10.1038/sj.onc.1209373
35. Sarkar S, Nagabhushan M, Soman CS, Tricker AR, Bhide SV (1989) Mutagenicity and carcinogenicity of smoked meat from Nagaland, a region of India prone to a high incidence of nasopharyngeal cancer. Carcinogenesis 10(4):733–736
36. Guo X, O'Brien SJ, Zeng Y, Nelson GW, Winkler CA (2008) GSTM1 and GSTT1 gene deletions and the risk for nasopharyngeal carcinoma in Han Chinese. Cancer Epidemiol Biomark Prev 17(7):1760–1763. https://doi.org/10.1158/1055-9965.EPI-08-0149
37. Jiang Y, Li N, Dong P, Zhang N, Sun Y, Han M, Wen J, Chen M (2011) Polymorphisms in GSTM1, GSTTI and GSTP1 and nasopharyngeal cancer in the east of China: a case-control study. Asian Pac J Cancer Prev 12(11):3097–3100
38. Wei Y, Zhou T, Lin H, Sun M, Wang D, Li H, Li B (2013) Significant associations between GSTM1/GSTT1 polymorphisms and nasopharyngeal cancer risk. Tumour Biol J Int Soc Oncodev Biol Med 34(2):887–894. https://doi.org/10.1007/s13277-012-0623-9
39. Cheng YJ, Chien YC, Hildesheim A, Hsu MM, Chen IH, Chuang J, Chang J, Ma YD, Luo CT, Hsu WL, Hsu HH, Huang H, Chang JF, Chen CJ, Yang CS (2003) No association between genetic polymorphisms of CYP1A1, GSTM1, GSTT1, GSTP1, NAT2, and nasopharyngeal carcinoma in Taiwan. Cancer Epidemiol Biomark Prev 12(2):179–180
40. Anantharaman D, Chaubal PM, Kannan S, Bhisey RA, Mahimkar MB (2007) Susceptibility to oral cancer by genetic polymorphisms at CYP1A1, GSTM1 and GSTT1 loci among Indians: tobacco exposure as a risk modulator. Carcinogenesis 28(7):1455–1462. https://doi.org/10.1093/carcin/bgm038
41. Soya SS, Vinod T, Reddy KS, Gopalakrishnan S, Adithan C (2007) Genetic polymorphisms of glutathione-S-transferase genes (GSTM1, GSTT1 and GSTP1) and upper aerodigestive tract cancer risk among smokers, tobacco chewers and alcoholics in an Indian population. Eur J Cancer 43(18):2698–2706. https://doi.org/10.1016/j.ejca.2007.07.006
42. Ghosh SK, Singh AS, Mondal R, Kapfo W, Khamo V, Singh YI (2014) Dysfunction of mitochondria due to environmental carcinogens in nasopharyngeal carcinoma in the ethnic group of Northeast Indian population. Tumour Biol J Int Soc Oncodev Biol Med 35(7):6715–6724. https://doi.org/10.1007/s13277-014-1897-x
43. Sam SS, Thomas V, Reddy SK, Surianarayanan G, Chandrasekaran A (2008) CYP1A1 polymorphisms and the risk of upper aerodigestive tract cancers in an Indian population. Head Neck 30(12):1566–1574. https://doi.org/10.1002/hed.20897
44. Chandirasekar R, Kumar BL, Sasikala K, Jayakumar R, Suresh K, Venkatesan R, Jacob R, Krishnapriya EK, Kavitha H, Ganesh GK (2014) Assessment of genotoxic and molecular mechanisms of cancer risk in smoking and smokeless tobacco users. Mutat Res Genet Toxicol Environ Mutagen 767C:21–27. https://doi.org/10.1016/j.mrgentox.2014.04.007
45. Bartsch H, Rojas M, Nair U, Nair J, Alexandrov K (1999) Genetic cancer susceptibility and DNA adducts: studies in smokers, tobacco chewers, and coke oven workers. Cancer Detect Prev 23(6):445–453
46. Sharma R, Ahuja M, Panda NK, Khullar M (2011) Interactions among genetic variants in tobacco metabolizing genes and smoking are associated with head and neck cancer susceptibility in north Indians. DNA Cell Biol 30(8):611–616. https://doi.org/10.1089/dna.2010.1184
47. Matthias C, Bockmuhl U, Jahnke V, Jones PW, Hayes JD, Alldersea J, Gilford J, Bailey L, Bath J, Worrall SF, Hand P, Fryer AA,

Strange RC (1998) Polymorphism in cytochrome P450 CYP2D6, CYP1A1, CYP2E1 and glutathione S-transferase, GSTM1, GSTM3, GSTT1 and susceptibility to tobacco-related cancers: studies in upper aerodigestive tract cancers. Pharmacogenetics 8(2):91–100

48. Varela-Lema L, Taioli E, Ruano-Ravina A, Barros-Dios JM, Anantharaman D, Benhamou S, Boccia S, Bhisey RA, Cadoni G, Capoluongo E, Chen CJ, Foulkes W, Goloni-Bertollo EM, Hatagima A, Hayes RB, Katoh T, Koifman S, Lazarus P, Manni JJ, Mahimkar M, Morita S, Park J, Park KK, Pavarino Bertelli EC, de Souza Fonseca Ribeiro EM, Roy B, Spitz MR, Strange RC, Wei Q, Ragin CC (2008) Meta-analysis and pooled analysis of GSTM1 and CYP1A1 polymorphisms and oral and pharyngeal cancers: a HuGE-GSEC review. Genet Med Off J Am Coll Med Genet 10(6): 369–384. https://doi.org/10.1097/GIM.0b013e3181770196
49. Choudhury JH, Ghosh SK (2015) Promoter hypermethylation profiling identifies subtypes of head and neck cancer with distinct viral, environmental, genetic and survival characteristics. PLoS One 10(6):e0129808. https://doi.org/10.1371/journal.pone.0129808
50. Laskar RS, Talukdar FR, Choudhury JH, Singh SA, Kundu S, Dhar B, Mondal R, Ghosh SK (2015) Association of HPV with genetic and epigenetic alterations in colorectal adenocarcinoma from Indian population. Tumour Biol J Int Soc Oncodev Biol Med 36(6):4661–4670. https://doi.org/10.1007/s13277-015-3114-y
51. Pietrusinski M, Kepczynski L, Jedrzejczyk A, Borkowska E, Traczyk-Borszynska M, Constantinou M, Kauzewski B, Borowiec M (2016) Detection of bladder cancer in urine sediments by a hypermethylation panel of selected tumor suppressor genes. Cancer Biomark Sect A Dis Mark. https://doi.org/10.3233/CBM-160673
52. Jiang W, Cai R, Chen QQ (2015) DNA methylation biomarkers for nasopharyngeal carcinoma: diagnostic and prognostic tools. Asian Pac J Cancer Prev 16(18):8059–8065
53. Lo KW, Chung GT, To KF (2012) Deciphering the molecular genetic basis of NPC through molecular, cytogenetic, and epigenetic approaches. Semin Cancer Biol 22(2):79–86. https://doi.org/10.1016/j.semcancer.2011.12.011
54. Dai W, Zheng H, Cheung AK, Lung ML (2016) Genetic and epigenetic landscape of nasopharyngeal carcinoma. Chinese. Clin Oncol 5(2):16. 10.21037/cco.2016.03.06
55. Talukdar FR, Ghosh SK, Laskar RS, Kannan R, Choudhury B, Bhowmik A (2015) Epigenetic pathogenesis of human papillomavirus in upper aerodigestive tract cancers. Mol Carcinog 54(11): 1387–1396. https://doi.org/10.1002/mc.22214
56. Lleras RA, Smith RV, Adrien LR, Schlecht NF, Burk RD, Harris TM, Childs G, Prystowsky MB, Belbin TJ (2013) Unique DNA methylation loci distinguish anatomic site and HPV status in head and neck squamous cell carcinoma. Clin Cancer Res 19(19):5444–5455. https://doi.org/10.1158/1078-0432.CCR-12-3280
57. Talukdar FR, Ghosh SK, Laskar RS, Mondal R (2013) Epigenetic, genetic and environmental interactions in esophageal squamous cell carcinoma from northeast India. PLoS One 8(4):e60996. https://doi.org/10.1371/journal.pone.0060996
58. Zhou L, Jiang W, Ren C, Yin Z, Feng X, Liu W, Tao Q, Yao K (2005) Frequent hypermethylation of RASSF1A and TSLC1, and high viral load of Epstein-Barr virus DNA in nasopharyngeal carcinoma and matched tumor-adjacent tissues. Neoplasia 7(9): 809–815
59. Tong JH, Tsang RK, Lo KW, Woo JK, Kwong J, Chan MW, Chang AR, van Hasselt CA, Huang DP, To KF (2002) Quantitative Epstein-Barr virus DNA analysis and detection of gene promoter hypermethylation in nasopharyngeal (NP) brushing samples from patients with NP carcinoma. Clin Cancer Res 8(8):2612–2619
60. Tsai CN, Tsai CL, Tse KP, Chang HY, Chang YS (2002) The Epstein-Barr virus oncogene product, latent membrane protein 1, induces the downregulation of E-cadherin gene expression via activation of DNA methyltransferases. Proc Natl Acad Sci U S A 99(15):10084–10089. https://doi.org/10.1073/pnas.152059399
61. Skalska L, White RE, Franz M, Ruhmann M, Allday MJ (2010) Epigenetic repression of p16(INK4A) by latent Epstein-Barr virus requires the interaction of EBNA3A and EBNA3C with CtBP. PLoS Pathog 6(6):e1000951. https://doi.org/10.1371/journal.ppat.1000951
62. Kulkarni V, Saranath D (2004) Concurrent hypermethylation of multiple regulatory genes in chewing tobacco associated oral squamous cell carcinomas and adjacent normal tissues. Oral Oncol 40(2):145–153
63. Hasegawa M, Nelson HH, Peters E, Ringstrom E, Posner M, Kelsey KT (2002) Patterns of gene promoter methylation in squamous cell cancer of the head and neck. Oncogene 21(27):4231–4236. https://doi.org/10.1038/sj.onc.1205528
64. Chang HW, Ling GS, Wei WI, Yuen AP (2004) Smoking and drinking can induce p15 methylation in the upper aerodigestive tract of healthy individuals and patients with head and neck squamous cell carcinoma. Cancer 101(1):125–132. https://doi.org/10.1002/cncr.20323
65. Ishida E, Nakamura M, Ikuta M, Shimada K, Matsuyoshi S, Kirita T, Konishi N (2005) Promotor hypermethylation of p14ARF is a key alteration for progression of oral squamous cell carcinoma. Oral Oncol 41(6):614–622. https://doi.org/10.1016/j.oraloncology.2005.02.003
66. Puri SK, Si L, Fan CY, Hanna E (2005) Aberrant promoter hypermethylation of multiple genes in head and neck squamous cell carcinoma. Am J Otolaryngol 26(1):12–17
67. Brait M, Ford JG, Papaiahgari S, Garza MA, Lee JI, Loyo M, Maldonado L, Begum S, McCaffrey L, Howerton M, Sidransky D, Emerson MR, Ahmed S, Williams CD, Hoque MO (2009) Association between lifestyle factors and CpG island methylation in a cancer-free population. Cancer Epidemiol Biomark Prev 18(11):2984–2991. https://doi.org/10.1158/1055-9965.EPI-08-1245
68. Lin RK, Hsieh YS, Lin P, Hsu HS, Chen CY, Tang YA, Lee CF, Wang YC (2010) The tobacco-specific carcinogen NNK induces DNA methyltransferase 1 accumulation and tumor suppressor gene hypermethylation in mice and lung cancer patients. J Clin Invest 120(2):521–532. https://doi.org/10.1172/JCI40706
69. Bonsch D, Lenz B, Fiszer R, Frieling H, Kornhuber J, Bleich S (2006) Lowered DNA methyltransferase (DNMT-3b) mRNA expression is associated with genomic DNA hypermethylation in patients with chronic alcoholism. J Neural Transm (Vienna) 113(9): 1299–1304. https://doi.org/10.1007/s00702-005-0413-2