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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

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ABSTRACT

The glutathione-S-transferase (GST) genes are involved in the detoxification of various carcinogens that increase the risk to upper aerodigestive tract (UADT) cancers. In the present study, 408 unrelated histopathologically confirmed cases and 220 population based controls, matched by age and gender, which belonged to the Tamilian population of south India were genotyped for polymorphisms in *GSTM1*, *GSTT1* and *GSTP1* using polymerase chain reaction (PCR) based methods. The multivariate logistic regression analyses demonstrated that *GSTT1* null genotype was significantly associated with increased risk for UADT cancers (odds ratio (OR) 2.5; 95% confidence intervals (CIs) 1.3–4.7). The combined effects of *GST* genes have shown that concurrent lack of *GSTM1* and *GSTT1* had a significantly increased risk (OR 4.6; 95% CI 1.3–15.6), while *GSTT1* null genotype along with *GSTP1* polymorphic variants further increased the cancer risk (OR 5.3; 95% CI 2.0–13.6). The most remarkable risk was seen among individuals carrying *GSTM1* null, *GSTT1* null genotypes and *GSTP1* polymorphic variants (OR 7.8; 95% CI 1.0–61.0). Tobacco chewers carrying *GSTM1* null genotype had an enhanced risk for UADT cancers. An enhanced risk among tobacco chewers and alcoholics (regular) was noted in individuals with *GSTT1* null genotype. Similarly, a significant interaction was observed among smokers (>40 pack-year (PY)) and tobacco chewers carrying *GSTP1* mutant genotypes. Although the null genotype of *GSTT1* is a strong predisposing risk factor for UADT cancers, we conclude that the significant gene–gene and gene–environment interactions of *GST* genes may confer a substantial risk to UADT cancers in the Tamilian population of south India.

1. Introduction

Cancer of the oral cavity, pharynx and larynx together constitutes upper aerodigestive tract (UADT) cancers.[1] It causes almost 4% of all the malignancies and 2% of deaths worldwide.[2] The global cancer statistics report of 2002 has shown that the worldwide incidence of UADT cancers was 643,869 and the total number of deaths reported due to UADT cancer was 351,740.[3] In India, it ranks first in males and third in females among all cancers.[4]

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Environmental exposures to genotoxic agents play an important role in causing human cancers. There is a cellular system for detoxification which protects the cells from DNA damage caused by various reactive substances.[5] Glutathione-*S*-transferases (GSTs) belong to a complex multigenic family of phase II metabolising enzymes. They have been found to be responsible for detoxification of a large number of electrophiles by conjugation reaction. This leads to the synthesis of mercapturic acids thereby facilitating the excretion of many xenobiotics.[6] The different isoenzymes of cytosolic GSTs are Mu, Theta, Pi, Sigma, Omega, Alpha and Zeta. It has been reported that deficient genotypes or polymorphism in GST Mu (M1), Theta (T1) and Pi (P1) contribute to increased susceptibility to various diseases.[7]

The detoxification of genotoxins including aromatic hydrocarbon epoxides and products of oxidative stress such as DNA hydroperoxides is catalysed by GSTM1.[8] The constituents of cigarette smoke such as alkyl halides[9] and cigarette smoke derived chemicals such as benzo(*a*)pyrene diol epoxide and acrolein[6] are catalysed by GSTT1. These carcinogens and toxins are found to be associated with increased susceptibility to UADT cancers.[10] The GSTP1 enzyme is widely expressed in tumour cells and is responsible for the detoxification of benzo(*a*)pyrene diol epoxide and acrolein present in cigarette smoke.[11]

The genes that code for GST isoenzymes, involved in the metabolic activation or detoxification of carcinogens, exhibit polymorphisms. Some of these polymorphisms have been found to affect the enzyme activity thereby influencing the individual cancer risk.[12] Among the *GST*s, *GSTM1* null, *GSTT1* null and the *GSTP1*-313 A/G substitution polymorphisms are widely investigated in diverse ethnic groups.[5] The functional consequence of the *GSTM1* and the *GSTT1* null genotypes is related to complete loss of enzyme activity. The *GSTP1* polymorphism at codon 105 where an adenosine to guanine (A–G) transition causes an isoleucine to valine substitution (*I105V*) that leads to decreased enzyme activity.[13] Therefore, the three genotypes for *GSTP1* are Ile/Ile (wild type; A/A), Ile/Val (heterozygous mutant; A/G) and Val/Val (homozygous mutant; G/G).

The genotype frequency of *GSTM1*, *GSTT1* and *GSTP1* genes has been documented in south India. The frequency of *GSTM1* null and *GSTT1* null was found to be 30% and 16.8%, respectively, whereas the frequency of both the *GSTM1* null and *GSTT1* null genotypes was found to be 4.6%.[14] In the Tamilian population, the genotype distributions of *GSTP1* were 44%, 47% and 9% for Ile/Ile, Ile/Val and Val/Val, respectively.[15] The frequency of *GSTM1* null in south Indians was significantly lower than that in Caucasians. The frequencies of both *GSTM1* and *GSTT1* null genotypes in south Indians were significantly lower than in Japanese.[14] The genotype distribution of *GSTP1*, *Ile/Ile* and *Ile/Val* in the Tamilian population varied significantly from Chinese but it was not significantly different from Caucasians.[15]

Polymorphisms of genes that code for these carcinogen detoxifying enzymes have shown variations in the prevalence between different ethnic and racial groups. About 10–65%[16] of individuals from different ethnic groups have been reported to possess null genotypes for *GSTM1* and *GSTT1*. When the *GSTT1* polymorphism alone was studied in different ethnic groups, it was found that the prevalence of the null genotype was highest among Chinese (64%), followed by Koreans (60%), African-Americans (22%) and Caucasians (20%), whereas the prevalence was lowest among Mexican-Americans (9.7%).[17]

The incidence of cancer mortality induced by smoking in African-American men was found to be higher compared to Caucasian men in the US population. It was reported that African-Americans smoke fewer cigarettes than Caucasians, hence higher rates of UADT cancers in African-Americans cannot be entirely attributed to smoking.[18] This indicates that genetic factors like *GST* polymorphism may also influence the susceptibility to UADT cancers. There are studies where association between *GSTM1* and *GSTT1* null genotypes (single gene or combined gene effect) and susceptibility to UADT cancers have been reported.[19–21] *GSTP1* polymorphisms have also been found to influence susceptibility to oral, pharyngeal and laryngeal carcinomas in a study done in Germans.[11]

Tamilians are an ethnic group from South Asia and they are ethnically, linguistically and culturally related to the other Dravidian population. The oldest Tamil communities are present in southern India and northeastern Sri Lanka. There are an estimated 70 million Tamilians around the world.[22] In view of the increased incidence of UADT cancers and a lack of data available on the association between *GST* genotypes and UADT cancer susceptibility in Tamilians, we investigated the association between *GSTM1* null, *GSTT1* null and the *GSTP1*-313 A/G polymorphism and risk to UADT cancers in the study population.

2. Subjects and methods

2.1. Subjects

Between December 2003 and July 2006, 408 patients (269 males and 139 females) having UADT cancers were recruited as cases. They were diagnosed at the Departments of ENT and Radiotherapy, JIPMER Hospital, Pondicherry, India. In all the cases, diagnosis of squamous cell carcinoma (SCC) of UADT cancers was confirmed by histopathologic examination. The control group consisted of 220 subjects (148 males and 72 females) without present or past history of any malignancies. The male:female ratio in both the groups was 2:1. The age and sex matched controls were selected randomly from those who came for treatment of various diseases other than malignancy at the same hospital and in the same period.

All the subjects were interviewed using a standardised questionnaire, regarding smoking habits, tobacco chewing habits and alcohol drinking history. The lifetime smoking consumption was expressed in pack-years. Data on frequency of alcohol consumption and tobacco chewing were also estimated. The study was approved by institutional ethics committee and written informed consent was obtained from all the subjects.

2.2. GSTM1 and GSTT1 genotyping

Five millilitres of venous blood was collected using ethylene diamine tetra acetic acid (EDTA) as anticoagulant. Genomic DNA was extracted from the peripheral leucocytes using stan-

dard phenol:chloroform method. *GSTM1* null and *GSTT1* null genotypes were simultaneously determined by means of a multiplex polymerase chain reaction (PCR) method.[23] The primers for *GSTM1* were 5′-GAACTCCCTGAAAAGCTAAAGC-3′ and 5′-GTTGGGCTCAAATATACGGTGG-3′; for *GSTT1*, the primers 5′-TTCCTTACTGGTCCTCACATCTC-3′ and 5′-TCACCG-GATCATGGCCAGCA-3′ were used. *Albumin* gene was amplified as an internal positive control using primers 5′-GCCCTCT-GCTAACAAGTCCTAC-3′ and 5′-GCCCTAAAAAGAAAATCGC-CAATC-3′. Agarose gel electrophoresis (1%) resolved amplified DNA fragments of 480-bp, 380-bp and 215-bp for *GSTT1*, *albumin* and *GSTM1*, respectively. Absence of DNA fragments of 215-bp and 480-bp indicates *GSTM1* null and *GSTT1* null genotypes, respectively (Fig. 1a).

2.3. *GSTP1 genotyping*

GSTP1 polymorphism was detected using the PCR-RFLP method.[24] The PCR mixture (50 μL) was prepared containing 30–50 ng of DNA, 5 μL of 10× buffer (500 mM KCl/100 mM Tris–HCl, pH 8.3/15 mM $MgCl_2$), 1 μL of 2.5 mM dNTPs, 0.5 μL of 20 pmol each of the forward primer, 5′-ACCCCAGGGCTC-TATGGGAA-3′ and reverse primer, 5′-TGAGGGCACAA-GAAGCCCCT-3′ and 2 U of *Taq* polymerase. PCR product of 10 μL was digested using BsmAI restriction enzyme. The digested PCR products were separated by electrophoresis using 8% polyacrylamide gel and stained with ethidium bromide. The genotypes were identified based on the size of DNA fragments. Digestion of the 176-bp amplicon resulted in either the retention of the 176-bp product or complete digestion to 93-bp and 83-bp fragments corresponding to individuals homozygous for the Ile/Ile or Val/Val genotypes, respectively. The presence of all three fragments corresponded to individuals heterozygous at codon 105 (Fig. 1b). Genotyping procedures were validated by sequencing of representative samples.

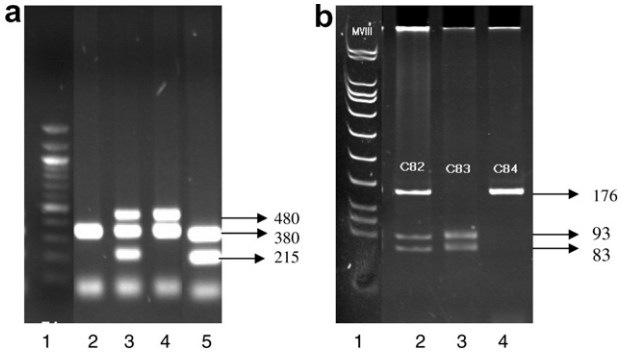

Fig. 1 – Representative gel pictures of GSTM1T1 (a) and GSTP1 (b) assays. (a) GSTM1T1 assay: lane 1: 100 bp DNA ladder; lane 2: absence of both GSTM1 and GSTT1; lane 3: presence of both GSTT1 and GSTM1; lane 4: presence of GSTT1; lane 5: presence of GSTM1. (b) GSTP1 (Ile/Val) assay: lane 1: molecular weight marker VIII; lane 2: Ile/Val; lane 3: Val/Val; lane 4: Ile/Ile genotype.

2.4. *Statistical analysis*

Statistical analysis was done using the Statistical Package for Social Sciences statistical software (SPSS Windows version release 13). The association between the *GSTM1*, *GSTT1*, *GSTP1* genotypes and UADT cancer risk was analysed by calculating the crude odds ratios (ORs) and 95% confidence intervals (95% CI) using the χ^2-test. The adjusted ORs were calculated using unconditional logistic regression analysis with the low risk genotype designated as the referent category. For analysing the gene–environment interactions, stratified variables (genotype X environmental factor) were generated and included in the logistic model simultaneously with appropriate indicator variables. The observed genotype frequencies were compared with expected frequencies to check for the Hardy–Weinberg equilibrium.

3. Results

The mean ages of cases and controls were 53.0 (±0.5) and 52.0 (±0.6), respectively. No significant difference in the gender distribution was observed between cases and controls. The number of smokers, alcoholics and tobacco chewers was more in cases than controls. The intensity of exposure and duration of the tobacco smoking, tobacco chewing and alcohol consumption showed a significant trend of dose–response effect that was associated with an increased risk for UADT cancers compared to the controls. Of the 408 patients, 187 (46%) had oral cavity cancer, 141 (34%) had pharyngeal cancer and 80 (20%) had cancer in laryngeal region (Table 1).

The frequency distributions of *GSTM1* null genotype in cases and controls were 27% and 22%, respectively. For *GSTT1* null genotype, the frequency distributions in cases and controls were 19% and 7.3%, respectively. The three genotypes of *GSTP1* viz. *Ile/Ile*, *Ile/Val* and Val/Val had a frequency distributions of 54%, 40%, 6.6% and 54%, 40%, 5.5% in cases and controls, respectively. The frequency of *Val* allele in cases and controls was found to be 0.26 and 0.25, respectively. When the association between *GST* genotypes and UADT cancer risk was analysed using unconditional logistic regression, *GSTT1* null genotype was associated with a nearly 2.5-fold risk for UADT cancers (OR, 2.5; 95% CI, 1.3–4.7). However, *GSTM1* and *GSTP1* did not show a significant risk to UADT cancers in the study population (Table 2). The observed *GST* genotype frequencies were consistent with Hardy–Weinberg equilibrium.

The risk associated with all the three high-risk *GST* genotypes (*GSTM1* null, *GSTT1* null and *GSTP1*, *Ile/Val* or *Val/Val*) compared to no risk genotypes (positive genotypes of *GSTM1*, *GSTT1* and *Ile/Ile* genotype of *GSTP1* designated as the reference group) was also investigated. The combined analyses of the *GST* genotypes showed a significantly increased risk for UADT cancers. *GSTT1* null genotype in individuals carrying *GSTM1* gene had a nearly 3-fold risk, whereas the risk was increased (4.6-fold) among carriers of null genotypes of both *GSTM1* and *GSTT1*. Although there was a reduction in the risk (1.7-fold) for UADT cancers in individuals with *GSTP1* ile/ile genotype and *GSTT1* null genotype, the risk was found to be increased (5.3-fold) in

Table 1 – Demographic characteristics of the study subjects

Sl. No.	Variables	Cases (408) *n* (%)	Control (220) *n* (%)	OR (95% CI)
1.	*Age, years* (mean ± SEM)	53.0 ± 0.49	52.0 ± 0.60	–
2.	*Gender*			
	Male	269 (66.0)	148 (67.0)	–
	Female	139 (34.0)	72 (33.0)	
3.	*Smoking, pack-years (PY)*[a]			
	0	150 (37.0)	141 (64.0)	1.0
	1–10	66 (16.2)	34 (15.5)	2.1 (1.1–4.0)*
	11–20	59 (14.5)	23 (10.5)	2.6 (1.3–5.4)**
	20–40	61 (15.0)	14 (6.4)	3.7 (1.7–8.0)***
	>40	72 (17.6)	8 (3.6)	7.9 (3.1–20.2)****
4.	*Tobacco chewers*[b]			
	Never	134 (33.0)	160 (73.0)	1.0
	Occasional	84 (20.0)	34 (15.5)	2.5 (1.5–4.2)****
	Regular	190 (47.0)	26 (11.8)	10.5 (6.3–17.3)****
5.	*Alcohol consumers*[c]			
	Never	181 (44.0)	146 (66.0)	1.0
	Occasional	65 (15.9)	44 (20.0)	0.65 (0.34–1.2)
	Regular	162 (40.0)	30 (13.6)	2.2 (1.2–4.1)*
6.	*Site of carcinoma*			
	Oral cavity	187 (46.0)	–	–
	Pharynx	141 (34.0)		
	Larynx	80 (20.0)		

a OR adjusted for age, gender, tobacco chewing and alcohol consumption.
b OR adjusted for age, gender, smoking and alcohol consumption.
c OR adjusted for age, gender, smoking and tobacco chewing.
* $P < 0.02$.
** $P < 0.01$.
*** $P < 0.001$.
**** $P < 0.0001$.

Table 2 – Distribution of GSTM1, GSTT1 and GSTP1 genotypes among the study subjects

Genotypes	Cases ($n = 408$)	Controls ($n = 220$)	OR[a] (95% CI)	*P* value	OR[b] (95% CI)	*P* value
GSTM1						
Present, *n* (%)	299 (73)	172 (78)	1.0		1.0	
Null, *n* (%)	109 (27)	48 (22)	1.3 (0.9–1.9)	0.21	1.2 (0.7–1.8)	0.52
GSTT1						
Present, *n* (%)	331 (81)	204 (93)	1.0		1.0	
Null, *n* (%)	77 (19)	16 (7.3)	2.9 (1.7–5.2)	0.0001	2.5 (1.3–4.7)	0.006
GSTP1						
Ile/Ile, *n* (%)	219 (54)	120 (54)	1.0		1.0	
Ile/Val, *n* (%)	162 (40)	88 (40)	1.0 (0.7–1.4)	0.96	0.85 (0.6–1.3)	0.44
Val/Val, *n* (%)	27 (6.6)	12 (5.5)	1.2 (0.6–2.5)	0.57	0.76 (0.3–1.8)	0.53

P value <0.05 is considered statistically significant.
a Crude OR.
b OR adjusted for age, gender, smoking, tobacco chewing and alcohol consumption.

patients having *GSTP1* polymorphic variants and *GSTT1* null genotype. However, a 7.8-fold increased risk was observed in individuals carrying *GSTM1* null, *GSTT1* null genotypes and *GSTP1* polymorphic variants (Table 3).

Further, to investigate the potential gene–environment interactions, analyses were carried out stratifying by smoking, tobacco chewing and alcohol consumption status. When the interactions between *GSTM1* and environmental factors were examined (Table 4), significant interactions were observed among the occasional and regular chewers carrying *GSTM1* null genotype. The OR was 3.6 (95% CI, 1.5–8.7) and 15.4 (95% CI, 5.8–41.0) in those carrying *GSTM1* deletion genotype versus 2.1 (95% CI, 1.2–3.8) and 9.4 (95% CI, 5.3–16.6) among those with the gene present. The observed ORs of 3.6 (occasional) and 15.4 (regular) were comparable with the expected multiplicative ORs of 2.9 and 12.2, respectively. However, no interaction between *GSTM1* and smoking and alcohol consumption was noticed.

Table 3 – Combined effects of GSTM1, GSTT1 and GSTP1 genotypes in the study subjects

Genotypes	Cases (n = 408)	Controls (n = 220)	OR[a] (95% CI)	P value
Double *GSTM1* and *GSTT1*, *n* (%)				
M1 (+/+) and T1 (+/+)	243 (59)	159 (72)	1.0	
M1 (–/–) and T1 (+/+)	88 (21)	45 (20)	1.3 (0.9–1.9)	0.26
M1 (+/+) and T1 (–/–)	56 (14.7)	13 (5.9)	2.8 (1.5–5.3)	0.001
M1 (–/–) and T1 (–/–)	21 (5.1)	3 (1.4)	4.6 (1.3–15.6)	0.008
Double *GSTM1* and *GSTP1*, *n* (%)				
M1 (+/+) and P1 (I/I)	170 (43)	97 (44)	1.0	
M1 (+/+) and P1 (I/V/or V/V)	129 (31)	75 (34)	0.98 (0.7–1.4)	0.92
M1 (–/–) and P1 (I/I)	49 (12)	23 (10.4)	1.2 (0.7–2.1)	0.58
M1 (–/–) and P1 (I/V/or V/V)	60 (14)	25 (11.4)	1.4 (0.8–2.3)	0.30
Double *GSTT1* and *GSTP1*, *n* (%)				
T1 (+/+) and P1 (I/I)	187 (46)	109 (50)	1.0	
T1 (+/+) and P1 (I/V/or V/V)	144 (35)	95 (43)	0.88 (0.6–1.3)	0.53
T1 (–/–) and P1 (I/I)	32 (7.8)	11 (5)	1.7 (0.8–3.5)	0.17
T1 (–/–) and P1 (I/V/or V/V)	45 (11.1)	5 (2.3)	5.3 (2.0–13.6)	<0.0001
Triple *GSTM1*, *GSTT1* and *GSTP1*, *n* (%)				
M1 and T1 (+/+) and P1 (I/I)	146 (36)	88 (40)	1.0	
M1 and T1 (+/+) and P1 (I/V/or V/V)	97 (24)	71 (32)	0.82 (0.6–1.2)	0.35
M1 (–/–), T1 (+/+) and P1 (I/I)	41 (10)	21 (9.5)	1.2 (0.7–2.1)	0.66
M1 (–/–), T1 (+/+) and P1 (I/V/or V/V)	47 (11.5)	24 (10.9)	1.2 (0.7–2.1)	0.58
M1 (+/+), T1 (–/–) and P1 (I/I)	24 (5.9)	9 (4.1)	1.6 (0.7–3.6)	0.33
M1 (+/+), T1 (–/–) and P1 (I/V/or V/V)	32 (7.8)	4 (1.8)	4.8 (1.7–14.1)	0.001
M1 (–/–), T1 (–/–) and P1 (I/I)	8 (2)	2 (0.9)	2.4 (0.5–11.6)	0.33
M1 (–/–), T1 (–/–) and P1 (I/V/or V/V)	13 (3.2)	1 (0.5)	7.8 (1.0–61.0)	0.02

M1 (+/+), *GSTM1* positive genotype; M1 (–/–), *GSTM1* null genotype; T1 (+/+), *GSTT1* positive genotype; T1 (–/–), *GSTT1* null genotype; P1 (I/I), *GSTP1* wild type (Ile/Ile); P1 (I/V/or V/V), *GSTP1* variant (Ile/Val or Val/Val).
a Crude OR (P value <0.05 is considered statistically significant).

Table 4 – Association between GSTM1 and UADT cancer, stratified by smoking, tobacco chewing and alcohol consumption status

Variables	*GSTM1* present			*GSTM1* null		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
Smoking[a], *pack-years (PY)*						
Non-smokers	112	107	1.0	38	31	1.1 (0.6–2.1)
1–10 PY	49	30	1.6 (0.8–3.2)	16	6	3.3 (1.1–10.4)
11–20 PY	45	17	3.2 (1.4–7.4)	15	6	1.3 (0.4–4.0)
21–40 PY	40	12	2.8 (1.2–6.5)	21	3	5.9 (1.5–23.1)
>40 PY	53	6	7.4 (2.6–21.4)	19	2	7.8 (1.5–40.3)
Tobacco chewing[b]						
Non-chewers	111	125	1.0	22	35	0.84 (0.5–1.6)
Occasional	56	27	2.1 (1.2–3.8)	29	8	3.6 (1.5–8.7)
Regular	132	20	9.4 (5.3–16.6)	58	5	15.4 (5.8–41.0)
Alcohol consumption[c]						
Non-alcoholics	126	113	1.0	55	33	1.3 (0.7–2.3)
Occasional	46	37	0.62 (0.3–1.3)	19	7	1.1 (0.4–3.3)
Regular	127	22	2.7 (1.3–5.4)	35	8	1.4 (0.5–3.9)

a OR adjusted for age, gender, tobacco chewing and alcohol consumption.
b OR adjusted for age, gender, smoking and alcohol consumption.
c OR adjusted for age, gender, smoking and tobacco chewing.

For *GSTT1* genotype, a statistically significant interaction was observed among tobacco chewers and the interaction was enhanced with increased exposure (Table 5). Among the occasional and regular chewers, the OR was 9.5 (95% CI, 2.7–33.7) and 52.0 (95% CI, 7.0–393.3), respectively, for individuals with *GSTT1* absent versus 1.8 (95% CI, 1.0–3.0) and 8.3 (95% CI, 4.9–13.9), respectively, for those with the *GSTT1* gene present. The observed ORs of 9.5 (occasional) and 52.0 (regular) were found to be higher than the expected multiplicative ORs of 6.2 and 26.0, respectively. A significant interaction was also observed among regular alcoholics carrying *GSTT1* null genotype in whom the OR was 10.6 (95% CI, 1.3–85.4) compared to an OR of 2.1 (95% CI, 1.1–3.9) in individuals having *GSTT1* gene. The OR of 10.6 was found to be significantly higher than the multiplicative OR of 5.3. There was no evidence of gene–environment interaction related to smoking.

We also observed a significant interaction of *GSTP1* polymorphism among smokers (>40 pack-year (PY)) (Table 6). The individuals polymorphic for *GSTP1* had an OR of 7.8 (95% CI, 2.0–30.7) whereas wild type carriers had an OR of 5.6 (95% CI, 1.7–17.9). The OR of 7.8 was found to be higher than the multiplicative OR of 6.6. Similar to *GSTT1* and *GSTM1*, an interaction was noted among occasional and regular chewers in those individuals polymorphic for *GSTP1*. For occasional and regular

Table 5 – Association between GSTT1 and UADT cancer, stratified by smoking, tobacco chewing and alcohol consumption status

Variables	*GSTT1* present			*GSTT1* null		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
Smoking[a], pack-years (PY)						
Non-smokers	123	131	1.0	28	7	3.9 (1.5–10.0)
1–10 PY	56	33	1.9 (1.0–3.5)	8	3	2.7 (0.6–11.8)
11–20 PY	43	20	2.3 (1.0–5.0)	17	3	5.5 (1.4–21.6)
21–40 PY	49	13	3.7 (1.7–8.2)	12	2	2.6 (0.5–13.7)
>40 PY	60	7	7.4 (2.7–19.7)	12	2	9.9 (1.1–87.6)
Tobacco chewing[b]						
Non-chewers	124	147	1.0	14	12	1.1 (0.5–2.8)
Occasional	59	32	1.8 (1.0–3.0)	25	3	9.5 (2.7–33.7)
Regular	148	25	8.3 (4.9–13.9)	38	1	52.0 (7.0–393.3)
Alcohol consumption[c]						
Non-alcoholics	146	134	1.0	36	12	2.2 (1.0–4.8)
Occasional	56	41	0.67 (0.4–1.3)	9	3	1.5 (0.3–6.6)
Regular	129	29	2.1 (1.1–3.9)	32	1	10.6 (1.3–85.4)

a OR adjusted for age, gender, tobacco chewing and alcohol consumption.
b OR adjusted for age, gender, smoking and alcohol consumption.
c OR adjusted for age, gender, smoking and tobacco chewing.

Table 6 – Association between GSTP1 and UADT cancer, stratified by smoking, tobacco chewing and alcohol consumption status

Variables	*GSTP1* ile/ile			*GSTP1* ile/val or val/val		
	Cases	Controls	OR (95% CI)	Cases	Controls	OR (95% CI)
Smoking[a], pack-years (PY)						
Non-smokers	79	74	1.0	72	64	0.78 (0.5–1.3)
1–10 PY	39	22	1.4 (0.7–3.0)	25	14	1.9 (0.8–4.8)
11–20 PY	31	11	2.9 (1.1–7.4)	29	12	1.7 (0.7–4.4)
21–40 PY	36	8	3.7 (1.4–9.8)	25	7	2.1 (0.7–5.9)
>40 PY	34	5	5.6 (1.7–17.9)	38	3	7.8 (2.0–30.7)
Tobacco chewing[b]						
Non-chewers	82	87	1.0	53	73	0.63 (0.4–1.1)
Occasional	44	19	1.9 (1.0–3.8)	40	16	2.1 (1.1–4.3)
Regular	95	14	7.6 (3.9–14.8)	94	11	10.5 (5.1–21.6)
Alcohol consumption[c]						
Non-alcoholics	100	85	1.0	81	61	0.84 (0.5–1.4)
Occasional	35	23	0.64 (0.3–1.4)	30	21	0.57 (0.3–1.3)
Regular	84	12	2.3 (1.0–5.3)	78	18	1.9 (0.9–3.9)

a OR adjusted for age, gender, tobacco chewing and alcohol consumption.
b OR adjusted for age, gender, smoking and alcohol consumption.
c OR adjusted for age, gender, smoking and tobacco chewing.

chewers, the ORs of 2.1 (95% CI, 1.1–4.3) and 10.5 (95% CI, 5.1–21.6) in carriers of *GSTP1* ile/val or val/val have shown to be significantly higher than the ORs of 1.9 (95% CI, 1.0–3.8) and 7.6 (95% CI, 3.9–14.8) in individuals homozygous for ile/ile genotype. The ORs of 2.1 and 10.5 were comparable with 2.1 and 8.8 for occasional and regular chewers, respectively, on the multiplicative scale. However, no interaction between *GSTP1* and alcohol consumption was noticed.

4. Discussion

The present study is the first report on the association between *GSTM1*, *GSTT1* and *GSTP1*-313A/G polymorphism and susceptibility to UADT cancers in the Tamilian population of south India and it is the largest Indian case–control study on UADT cancers for *GST* genotypes. When the influence of the three genes on the UADT cancer susceptibility was analysed separately, we found a 2.5-fold increased risk for UADT cancers in cases carrying *GSTT1* null genotype, whereas *GSTM1* null and variant genotypes of *GSTP1* were not significantly associated with UADT cancer risk. Absence of GSTT1 isoenzyme in patients with *GSTT1* null genotypes would have resulted in the failure of the detoxification of carcinogens and environmental toxins, thereby increasing the risk for UADT cancers due to smoking, alcohol and tobacco chewing.

GSTs are mainly involved in the detoxification of a wide variety of potentially toxic and carcinogenic electrophiles by conjugating with glutathione. They are also involved in the deactivation of oxidative metabolites of endogenous or exogenous carcinogenic agents (industrial chemicals, dietary compound, tobacco products, drugs and environmental carcinogens) that are probably associated with UADT cancer risk.[25] Individuals with altered form of the enzymes (null genotypes of *GSTM1* or *GSTT1* and the *Ile/Val* or *Val/Val* genotypes of *GSTP1*) cannot detoxify the activated carcinogen leading to progression of cancer. Induction of other enzymes and proteins important for cellular function, e.g. DNA repair[26] is also modulated by *GST*s. Hence, they are important in cancer susceptibility and also for maintaining cellular genomic integrity.

In the complex polygenic disease such as UADT cancers, it is likely that genetic susceptibility is dependent on the action of several gene polymorphisms operating in concert. Polymorphisms in individual genes may only impart to a small extent, and it is likely that the cumulative effect of many polymorphisms will be important in its pathogenesis. Therefore, we analysed the *GSTT1*, *GSTM1* and *GSTP1* genes to determine whether the genotypes in combination alter the cancer susceptibility. The risk associated with *GSTT1* null genotype in the presence, as well as in the absence, of *GSTM1* genotype depicts the independent involvement of *GSTT1* gene deletion in the etiology of UADT cancers. The decreased risk noticed when *GSTP1* wild genotype combined with *GSTT1* null genotype might be due to the fact that tobacco or alcohol derived carcinogens and toxins are multiple substrates for GSTP1. This was confirmed by the increased risk observed among carriers of *GSTP1* polymorphic variants along with *GSTT1* null genotype. In addition, a significant association was observed for concurrent deletion of the *GSTM1*, *GSTT1* genes and mutant genotypes of *GSTP1* indicating that individuals having a defective genotype for more than one of these genes would therefore be at greater risk. Similar results were reported for various combinations of *GST* polymorphisms and susceptibility to oral leukoplakia,[27] larynx cancers[28] and head and neck cancers.[29]

The environmental factors like smoking, alcohol, tobacco chewing and occupational exposure to toxins and carcinogens are responsible for almost 90% of all cancers. The involvement of these factors along with the genetic polymorphism has a major role in the etiology of the UADT cancers.[30] For any given environmental exposure, individual differences in susceptibility might have a genetic basis. Genetic variability in metabolic activation and detoxification of environmental carcinogens may partially explain host susceptibility to chemically induced cancers.[31] The present study shows that gene–gene interaction may also contribute to the risk of developing UADT cancers.

Significant gene–environment interactions that further modify the susceptibility to UADT cancers were noted. The strongest joint effect was observed among the tobacco chewers who were polymorphic for *GST* genes under study. In our study, it was noticed that chewers used tobacco in the form of betel quid (BQ) that consists of betel leaf (*Piper betle* L.), betel or areca nut (*Areca catechu* L.), slaked lime [$Ca(OH)_2$], catechu (*Acacia catechu* L.) and tobacco. The chewing of tobacco with BQ results in exposure to carcinogenic tobacco specific nitrosamines (TSNA) and nitrosamines derived from areca nut alkaloids. It has also been reported that chewing of BQ generates a high amount of reactive oxygen species (ROS) in the mouth, which has been implicated in multistage carcinogenesis. Thus, TSNA and ROS are the major genotoxic agents involved in chewing related UADT cancers.[27,32]

Our findings did not suggest a strong interaction between smoking, *GSTT1* and *GSTM1* null genotypes. Two studies,[33,34] conducted in eastern (Kolkata) and western (Mumbai) parts of India, reported that *GSTM1* null genotype is a risk factor for the development of oral cancer among tobacco users. In another Indian study done in Keralites[35] (a south Indian population), neither *GSTM1* null nor *GSTT1* null significantly contributed to the susceptibility to oral cavity carcinoma among tobacco users. However, in the present study, a significant interaction was noticed only among smokers (>40 PY) carrying *GSTP1* polymorphism. The Bidi (a type of cigarette) is the most prevalent form of smoking in India. In our study population, tobacco was smoked as cigarettes or in the form of bidi, a native cigarette-like stick that consists of tobacco wrapped in a tendu or temburni leaf. The GSTP1 enzyme that is involved in the detoxification of benzo(*a*)pyrene diol epoxide and acrolein present in cigarette smoke[11] might have enhanced the UADT cancer risk among smokers (>40 PY) carrying *GSTP1* polymorphic genotypes. A German study reported that polymorphism at *GSTP1* mediates susceptibility to squamous cell carcinoma of the upper aerodigestive tract as it was found that there was a decreased frequency of Ile/Ile genotype in cancer patients compared to controls.[11] Our study did not show a significant association between *GSTP1*-313A/G polymorphism and UADT cancer risk, but in the presence of potential hazardous environmental factors and genotypes, a significant gene–environment as well as gene–gene interaction was observed among the carriers of *GSTP1*

polymorphisms, which might explain the vital role of the gene that contributes to UADT carcinogenesis in our study population.

The interaction observed among regular alcoholics carrying *GSTT1* null genotype was in agreement with an Indian study,[36] where regular alcoholics carrying *GSTT1* null had an almost 3-fold increased risk for the development of multiple primary neoplasms in UADT cancers. The chronic use of alcohol consumption has been implicated as a risk factor for cancers of UADT where it acts as a solvent and enhances the penetration of carcinogens into the mucosa.[37,38] The *GSTT1* deletion among the regular alcoholics would have exacerbated the UADT cancer pathogenesis compared to other *GST* genes.

In a study carried out in the Chinese population, smokers, alcoholics and tobacco chewers having null genotypes of *GSTM1* and/or *GSTT1* had a significantly increased oral cancer risk compared with those who had non-null genotypes of both *GSTM1* and *GSTT1*.[39] A study carried out in US smokers has shown that *GSTM1* and *GSTT1* null genotypes are independent risk factors for squamous cell carcinoma of head and neck and depicted as markers for genetic susceptibility to tobacco-induced tumourigenesis.[18] Our results partially support these findings as we could observe only a few interactions associated with *GSTM1* and *GSTT1* genes, which may be explained on the basis of inter-ethnic differences.

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

To our knowledge, this is the first genetic study of UADT cancers carried out in the Tamilian population of south India, and showed that the null genotype of *GSTT1* is a strong predisposing risk factor for UADT cancers. The combined effects of *GST* mutant genotypes (gene–gene interaction) indicate the risk for developing UADT cancers. Further, the interaction between the *GSTM1* null, *GSTT1* null or *GSTP1* variant genotypes and the environmental factors (gene–environment interaction) significantly modifies the UADT cancer risk in the study population.

Conflict of interest statement

None declared.

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