Genetic Polymorphisms in *CYP1A1*, *GSTM1*, *GSTP1*, *GSTT1*, *NAT1* and *NAT2* and Oral Cavity Cancer Risk among Filipinos

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ABSTRACT

Polymorphisms in metabolic genes have been shown to modulate susceptibility to oral cavity cancer. Cases (n=176) and controls (n=317) from the Filipino population were genotyped for selected polymorphisms in *CYP1A1*, *GSTM1*, *GSTP1*, *GSTT1*, *NAT1* and *NAT2*. Medical and diet histories, occupational exposure and demographic data were also collected for all subjects. The *CYP1A1m1/m1* genotype is protective against oral cancer, while being homozygous for the *GSTP1* c.313G genotype and heterozygous for the *NAT1*10* allele are significant risk factors for oral cancer. Genetic subgroup analyses suggest that the risk conferred by homozygosity for the *GSTP1* variant was only significant among *NAT1*10* homozygotes and non-homozygotes for the *CYP1A1 m1* allele. The risk from heterozygosity for the *NAT1*10* allele was limited to subjects who were not homozygous for the *GSTP1* c.313G polymorphism. After adjusting for environmental variables, the homozygous *GSTP1 c.313G* genotype remained a significant oral cancer risk modifier, together with environmental risk factors, such as smoking, passive smoking, inverted smoking and tobacco chewing, and environmental protective factors, i.e. moderate consumption of fish sauce (*patis*) and shrimp paste (*bagoong*). The *GSTP1 c.313G* polymorphism increases susceptibility for oral cavity cancer in the Filipino population.

Key Words: Filipinos, genetic polymorphisms, metabolic enzyme, oral cavity cancer, risk factor, susceptibility

Introduction

Worldwide about 400,000 cases of oral cavity and pharyngeal cancer and 160,000 cases of laryngeal cancer are reported annually.[1] In the Philippines, the oral cavity ranks as the 15th most common cancer site among women (age-standardized rate incidence: 1.7), 10th among men (age-standardized rate incidence: 2.6), and 15th for both sexes (age-standardized rate incidence: 2.1).[2]

Risk factors for oral cavity cancer include alcohol consumption, smoking, tobacco and betel nut chewing, as well as occupational exposure to certain chemicals.[1] However, the carcinogenic risk attributed to these compounds can also be modified by genetic susceptibility factors in the host, such as genetic polymorphisms, that potentially predict cancer risk.[3] Phase I and Phase II metabolizing enzyme genes encode proteins that catalyze either the effective detoxification of various endogenous and exogenous substrates into harmless compounds, or their metabolic activation into toxic and carcinogenic products. Many of these genes are functionally polymorphic due to different allelic variants. The phenotypic differences in these enzymes could, in turn, result in varied susceptibility to cancer among individuals.[4] Allelic variants in genes from the Cytochrome P450, Glutathione-S-Transferase and N-acetyltransferase superfamilies can significantly influence risk of developing oral cavity cancer.[5,6,7]

Cytochrome P450 (CYP) enzymes are responsible for catalyzing the carcinogenic activation of various endobiotics (i.e. steroids and fatty acids) and xenobiotics found in the environment or our diet (i.e. polycyclic aromatic hydrocarbons, aromatic amines and mycotoxins).[8,9] In some cases, CYP enzymes are responsible for detoxifying certain metabolites.[10] Among the CYP genes, *CYP1A1* variants have been associated with lung, esophageal, and head and neck cancers. There are two *CYP1A1* polymorphisms that have been found to increase the enzyme's inducibility and catalytic activity: the p.Ile462Val polymorphism in *CYP1A1* exon 7, called the *m2* allele (g.4889A>G); and the T-to-C transition in the 3′ non-coding region of the gene—detectable by *Msp*I restriction, named the *m1* allele (g.6235T>C).[5,8,11,12,13]

The Gluthathione S-transferase (GST) genes are primarily involved in cellular detoxification by preventing

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the attack of reactive electrophiles on macromolecules.[14] These multifunctional phase II enzymatic proteins catalyze the conjugation of glutathione to carcinogens and their reactive intermediates, rendering them more water-soluble to facilitate subsequent excretion.[15,16] Increased cancer risk has been associated with GST genotypes harboring homozygous null alleles, as in the case of *GSTM1* and *GSTT1*, and those that code for low activity variants, which has been observed for *GSTP1*.[17,18,19]

The N-acetyltransferase genes *NAT1* and *NAT2* perform N-acetylation, O-acetylation, and N,O-acetyltransfer which activate or deactivate aromatic amines; Activation of these species results in the formation of acetoxy esters that decompose into highly electrophilic aryl nitrenium ions capable of initiating carcinogenesis. Polymorphic variants of *NAT1* and *NAT2* have different rates of acetylation.[20,21] Different alleles of both genes, in particular the *NAT1*10* allele and the *NAT2* slow acetylator genotypes, have been shown to confer increased risk for oral cavity cancer.[22,23,24] In a recent meta-analysis, NAT2 slow acetylator polymorphisms have been found to potentially increase oral cancer risk among Asians but not among Caucasians or other races.[25]

To our knowledge, this is the first report of the association between metabolic enzyme gene polymorphisms and oral cavity cancer within the Filipino population. Specifically the Phase I and II metabolic enzyme genes *CYP1A1, GSTM1, GSTP1, GSTT1, NAT1* and *NAT2* were genotyped in oral cavity cancer patients and controls and the cancer risk conferred by single genes and multiple genes taken together was estimated. We also investigated the interaction of these polymorphisms with environmental risk factors for oral cavity cancer.

Materials and Methods

Subject Population

Prior to study initiation, approval was obtained from Ethics Review Committee of the Research Implementation and Development Office, College of Medicine, University of the Philippines Manila. Informed consent was obtained from all subjects. A total of 176 cases with histopathologically confirmed carcinoma of the oral cavity and 317 healthy controls were enrolled in the case-control study. The patients were recruited from four tertiary hospitals (Philippine General Hospital (PGH), Jose R. Reyes Memorial Medical Center (JRRMMC), East Avenue Medical Center (EAMC) and Ospital ng Maynila Medical Center (OMMC) from June 2002 to September 2008. The inclusion criteria was the presence of histopathologically confirmed carcinoma of the oral cavity (any age, any stage, any Eastern Cooperative Oncology Group) and no history of chemotherapy or radiotherapy prior to study enrolment. The inclusion criterion for the controls was the absence of a prior history of and clinical signs of cancer. The controls (age-5 years-interval-matched; sex-matched) were randomly selected from the same hospitals as the cases during the same time period.

Data Collection

All individuals were interviewed by trained health workers using a questionnaire and standardized interview and measurement techniques. Information on multiple variables were collected, including age, gender, occupation, tobacco and betel nut chewing habits, family history of head and neck cancer, oral contraceptive use, diet (consumption of: alcohol, canned meat, fish sauce (*patis*), shrimp paste (*bagoong*), vegetables, scalding hot food, preserved foods, smoked foods, salted foods), and occupational exposure (exposure to: moldy food, pesticides, vinyl chloride, benzene, UV sunlight, coal carbonization, and wood dust). The questionnaire and the interview technique were pre-tested among a group of Filipino patients and were modified accordingly. Blinding of interviewers as to case-control status could not be completely done since some tumors were visible.

DNA Isolation

Four milliliters of peripheral blood was collected from each subject for molecular genotyping. Genomic DNA was extracted from whole blood samples of recruited cases and controls using the QIAamp® Blood Midi Kit Spin Protocol (Qiagen GmbH, Hilden, Germany). The DNA extracts were subsequently stored at – 20°C.

Molecular Genotyping

Polymorphisms for *CYP1A1, GSTM1, GSTT1, GSTP1, NAT1* and *NAT2* genes were detected using polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (RFLP). PCR and RFLP products were visualized using agarose gel electrophoresis. Genotyping methods by PCR-RFLP were verified by direct sequencing.

CYP1A1.The *m1* allele (g.6235T>C) at the 3′ flanking region of the gene was ascertained with *MspI* while the *m2* allele at exon 7 was detected with *NcoI* using PCR-RFLP as previously described.[8,6,27]

***GSTM1* and *GSTT1*.** The presence of at least one allele of *GSTM1* and *GSTT1* was determined using PCR.[8] As an internal control, exon 7 of the *CYP1A1* gene was co-amplified for each reaction using previously published primers.[28]

GSTP1. The c.313A>G polymorphism in exon 5 was ascertained using PCR-RFLP with *Alw*26I.[18]

NAT1. Detection of *NAT1* alleles was performed using PCR-RFLP with *Mbo*II for *NAT1*4, and NAT1*11*. Allele-specific

PCR was performed using *NAT1*3*-specific and *NAT1*10*-specific primers to distinguish between the two alleles.[29] As an internal control for allele-specific PCR, β-globin was co-amplified using previously published primers.[8]

NAT2. Detection of *NAT2*alleles was performed using the PCR-RFLP strategy by Hubbard et al.[30]

Statistical Analysis

For the environmental variables, statistical analysis was done using Stata Program version 9.0, while SPSS 14.0 software was used for statistical analysis of genetic variables. Age-and sex-matched pairs were analyzed including multiple controls per case within an age-group. Comparison of characteristics between groups was carried out through χ^2 tests for genetic variables, McNemar's test for environmental variables, and independent t-tests for continuous (environmental) variables. Univariate conditional logistic regression analyses for environmental and genetic variables were initially carried out separately using simple conditional logistic regression to assess the significance of each independent variable or risk factor for cancer by cancer site. All environmental factors that were significant at 0.2 level in the univariate analyses were included in the multivariate conditional logistic regression model. All environmental factors that were significant in the multivariate analysis were included in multivariate logistic regression with statistically significant genetic variables to test for gene-environment interactions.

For diallelic genes *GSTP1*, *CYP1A1* and *CYP2E1* genotypes were tested for Hardy-Weinberg Equilibrium (HWE). Only four of 26 known *NAT1* alleles and six of 62 known *NAT2* alleles were tested, thus genotype frequencies for both genes are expected to deviate from HWE.[31]

Results

A total of 176 cases and 317 controls were available for study (Table 1). There were 210 males (42.6%) and 283 females (57.4%). Median age range for the whole group was 50-54 years old, 45-49 for the controls and 55-59 for the cases. Age-matched logistic regression of environmental factors revealed that current smoking, former smoking, passive smoking, inverted smoking, tobacco chewing, betel quid chewing, consumption of scalding food (>5 days/month), consumption of salted food (>5 days/month), UV sunlight exposure (≥7/month) are significant risk factors for oral cancer; while canned meat consumption (daily to 2x/month), using fish sauce or *patis* (daily to 2x/month) and shrimp paste or *bagoong* (daily to 2x/month) were significant protective factors (Table 2). After multivariate analysis, current smoking (OR 1.99; 95%CI: 1.20, 3.31), passive smoking (OR 2.81; 95%CI: 1.57, 5.06), inverted smoking (OR 3.22; 95%CI: 1.28, 8.08) and chewing tobacco (OR 5.16; 95%CI: 1.37, 19.50) were found to increase susceptibility for oral cancer, while use of shrimp paste (*bagoong*) (OR 0.48; 95%CI: 0.27, 0.84) and fish sauce (*patis)* (OR 0.44; 95%CI: 0.25, 0.78) were significant protective factors (Table 2).

Table 1. Age and sex distribution of oral cavity cancer cases and controls

Age	Control		Case		Total
	Male	Female	Male	Female	
<20	4	8	0	0	12
20-24	8	12	3	0	23
25-29	8	6	0	0	14
30-34	10	17	4	3	34
35-39	13	13	3	1	30
40-44	18	22	7	3	50
45-49	9	26	10	5	50
50-54	13	33	19	9	74
55-59	9	16	13	11	49
60-64	10	16	12	15	53
65-69	6	13	9	15	43
70-74	8	8	5	7	28
75-79	2	3	4	11	20
>79	2	4	1	6	13
Total	**120**	**197**	**90**	**86**	**493**

Table 2. Age- and sex-matched univariate logistic regression analysis for environmental factors and oral cavity cancer[a]

Variable	OR	OR 95% CI
Nonsmoker	1.00	
Current smoker[b]	3.12	1.79 ,5.43
Ex-smoker	2.26	1.28, 4.00
Chew tobacco[b]	6.04	1.73, 21.07
Inverted cigarette smoker[b]	4.45	1.97, 10.04
Passive smoker[b]	3.97	2.32, 6.81
Non-drinker, alcohol	1.00	
Current drinker, alcohol	1.19	0.67, 2.11
Ex-drinker, alcohol	0.91	0.48, 1.74
Canned meat eater (daily-2/month)	0.42	0.26, 0.67
Fish sauce (*patis*) user (daily-2/month)[b]	0.24	0.15, 0.37
Shrimp paste (*bagoong*) user (daily-2/month)[b]	0.26	0.17, 0.40
Vegetable eater (≥ 1/week)	0.64	0.30, 1.36
Family history head & neck cancer, 1st degree	0.76	0.12, 4.80
Scalding hot-food taker (> 5 days/month)	1.73	1.13, 2.65
Preserved food (nitrite-treated) eater (>5 days/month)	0.69	0.44, 1.08
Smoked food eater (> 5 days/month)	1.52	0.95, 2.44
Salted food eater (> 5 days/month)	2.71	1.61, 4.56
Moldy food exposure (≥ 1/ month)	0.69	0.46, 1.05
Oral contraceptive use (≥ 1/ year)	0.56	0.22, 1.42
Pesticide exposure (≥ 1/week)	1.64	0.79, 3.39
Vinyl chloride occupational exposure	1.00	0.24, 4.22
Benzene occupational exposure	0.69	0.16, 2.96
UV sunlight exposure (≥ 7/month)	2.01	1.23, 3.29
Wood dust occupational exposure	2.63	0.96, 7.20
Betel quid chewing	6.94	2.01, 23.93

[a]*Previously published in Acta Medica Philippina. Source: Ngelangel A, Javelosa MA, Cutiongco-de la Paz, EM and The Philippine Cancer Genetics Study Group. Epidemiological Risk Factors for Cancers of the Lung, Breast, Colon-rectum & Oral cavity: A Case-Control Study in the Philippines. Acta Medica Philippina 2009; 43(4):29-34*

[b]*Environmental factors that remained significant after age and sex-matched multivariate logistic regression: current smoking (OR 1.99; 95% CI 1.20-3.31), passive smoking (OR 2.81; 95% CI 1.57-5.06), tobacco chewing (OR 5.16; 95% CI 1.37-19.5), and inverted smoking (OR 3.22; 95% CI 1.28-8.08), consumption of shrimp paste (bagoong), daily-2/month (OR 0.48; 95% CI 0.27-0.84), and consumption of fish sauce (patis), daily-2/month (OR 0.44; 95% CI 0.25-0.78)*

For the genetic factors, age- and sex-adjusted univariate logistic regression showed that cancer risk increases two-fold with the *GSTP1* c.313A>G homozygous genotype (OR 2.07; 95% CI: 1.03, 4.16) and the *GSTP1* c.313A>G allele, recessive model (OR 2.26; 95%CI: 1.15, 4.44) (Table 3). In multivariate analysis, the *GSTP1* variant homozygous genotype remains a significant risk factor (OR 2.98; 95%CI: 1.35, 6.57) (Table 4). Among the NAT genes, univariate analysis (dominant model) identified only the *NAT1*10* allele (OR 1.78; 95% CI 1.05-3.04) as a significant risk factor, while the *NAT1*10* heterozygote genotype increased cancer susceptibility based on both univariate (OR 1.86; 95% CI 1.04-3.34) (Table 3) and multivariate (OR 2.15; 95% CI 1.16-3.96) analyses (Table 4). Among the polymorphisms studied, only *CYP1A1* polymorphisms were found to protect against oral cavity cancer. From univariate analyses, the *CYP1A1m1/m1* genotype (OR 0.50; 95%CI: 0.28, 0.88) and *m1* allele (dominant model) (OR 0.66; 95%CI: 0.45, 0.97**)**, as well as heterozygosity for the *CYP1A1 m2* allele (OR 0.66; 95%CI: 0.44, 0.99) reduced risk for oral cancer (Table 3). However, after multivariate analyses, only the *CYP1A1m1/m1* genotype remained significantly protective (OR 0.45; 95% CI 0.24-0.85) (Table 4).

Table 3. Age- and sex-adjusted univariate logistic regression analyses by oral cavity cancer status

Variable[a]	OR	OR 95% CI
GSTM1 null	0.88	0.60, 1.29
GSTT1 null	1.02	0.70, 1.48
GSTP1 c.313A>G homozygote	2.07	1.03, 4.16
GSTP1 c.313 A>G heterozygote	0.82	0.55, 1.21
GSTP1 c.313 A>G allele (dominant)	0.96	0.66, 1.38
GSTP1 c.313 A>G allele (recessive)	2.26	1.15, 4.44
CYP1A1 g. 6235T>C (*m1*) homozygote	0.50	0.28, 0.88
CYP1A1 g. 6235 T>C (*m1*) heterozygote	0.72	0.48, 1.08
CYP1A1 g. 6235 T>C (*m1*) allele (dominant)	0.66	0.45, 0.97
CYP1A1 g. 6235 T>C (*m1*) allele (recessive)	0.60	0.36, 1.02
CYP1A1 g.4889A>G (*m2*) homozygote	0.84	0.33, 2.17
CYP1A1 g.4889A>G (*m2*) heterozygote	0.66	0.44, 0.99
CYP1A1 g.4889A>G (*m2*) allele (dominant)	0.66	0.46, 1.00
CYP1A1 g.4889A>G (*m2*) allele (recessive)	0.97	0.38, 2.48
*NAT1*3* allele	0.29	0.07, 1.33
*NAT1*4* allele	0.87	0.60, 1.27
*NAT1*10* homozygote	1.69	0.94, 3.02
*NAT1*10* heterozygote	1.86	1.04, 3.34
*NAT1*10* allele (dominant)	1.78	1.05, 3.04
*NAT1*10* allele (recessive)	1.06	0.20, 1.55
*NAT1*11* allele	2.06	0.86, 4.95
*NAT2*4* allele	1.06	0.72, 1.57
*NAT2*5A* allele	1.80	0.11, 28.9
*NAT2*5B* allele	1.39	0.77, 2.53
*NAT2*5C* allele	0.22	0.03, 1.77
*NAT2*6B* allele	1.04	0.72, 1.52
*NAT2*7A*allele	1.00	0.67, 1.50

[a]*Formula: Oral cavity cancer status ~ constant + βVariable*Variable*

Subgroup analyses of significant genotypes were also carried out. Comparing samples by *GSTP1* genotype shows that heterozygosity for the *NAT1*10* allele confers risk among those who have only one or no copy of the *GSTP1* c.313A>G allele (OR 2.2; 95% CI 1.18-4.10) that is not seen among subjects that are homozygous for the *GSTP1* c.313A>G allele (Table 5). However, when grouped according to *NAT1*10* status, the data suggests that the increased oral cancer risk conferred by *GSTP1* c.313A>G homozygote genotype is significant among subjects who are homozygous for the *NAT1*10* risk allele (OR 8.8; 95% CI 2.4-32.34) compared to other *NAT1* genotypes (Table 5). When genotype subgroups were compared according to *CYP1A1* genotype, susceptibility for oral cavity cancer due to *GSTP1* c.313A>G homozygote genotype is more significant among subjects who have no or only one copy of the *CYP1A1* g.6235T>C allele (OR 3.81; 95% CI 1.6-9.04) (Table 5).

Table 4. Multivariate logistic regression analyses of genetic factors, all genotypes

Variable[a]	OR	OR 95%CI
Constant	--	--
Age	1.06	1.04, 1.08
Male sex	2.11	1.39, 3.21
GSTP1 wildtype		
GSTP1 c.313A>G heterozygote	0.90	0.58, 1.38
GSTP1 c.313A>G homozygote	2.98	1.35, 6.57
CYP1A1 wildtype		
CYP1A1 g.6235T>C heterozygote	0.73	0.46, 1.15
CYP1A1 g.6235T>C homozygote	0.45	0.24, 0.85
Non-*NAT1*10* genotype		
*NAT1*10* heterozygote	2.15	1.16, 3.96
*NAT1*10* homozygote	1.85	0.98, 3.48

[a]*Model parameters: N = 490; -2LL = 542.256; R^2 = 0.18; CYP1A1 c.4889A>G was removed from the model after backward logistic regression.*

Once environmental variables were combined with genetic polymorphisms found to be significant in the multivariate logistic regression model, only the homozygote *GSTP1* c.313A>G was shown to confer a significant three-fold risk (OR 3.16; 95% CI 1.32-7.59) for oral cavity cancer, along with environmental factors such as smoking (OR 2.77; 95% CI 1.49-5.14), passive smoking (OR 2.79; 95% CI 1.55-5.00), inverted smoking (OR 2.78; 95% CI 1.06-7.32) and tobacco chewing (OR 4.50; 95% CI 1.19-17.05) (Table 6). From the model, fish sauce (*patis*) consumption of at most 1x/month (OR 0.42; 95% CI 0.22-0.81), and shrimp paste (*bagoong*) consumption of at most 1x/month (OR 0.44; 95% CI 0.23-0.84) were still significantly protective (Table 6).

Table 5. Age- and sex-adjusted multivariate logistic regression analyses when grouped according to genotype

Genotype Group	N	Genotype Tested	OR	OR 95%CI
GSTP1 c.313A>G non-homozygote	353	*NAT1*10* Heterozygote	2.20	1.18, 4.10
CYP1A1 g.6235T>C non-homozygote	404	*GSTP1* c.313A>G homozygote	3.81	1.60, 9.04
*NAT1*10* Homozygote	176	*GSTP1* c.313A>G homozygote	8.80	2.40, 32.34

Table 6. Multivariate logistic regression analyses of genetic and environmental factors

Variable[a]	OR	OR 95%CI
Constant	--	--
Age	1.06	1.04, 1.07
Male sex	1.74	0.99, 3.07
GSTP1 c.313A>G homozygote	3.16	1.32, 7.59
Non-smoker		
Current smoker	2.77	1.49, 5.14
Ex-smoker	1.88	0.95, 3.73
Passive smoker	2.79	1.55, 5.00
Tobacco chew	4.50	1.19, 17.05
Inverted smoking	2.78	1.06, 7.32
Non-fish sauce (*patis*)-eater		
At most 1x/month	0.42	0.22, 0.81
1x/week – 2x/day	0.62	0.32, 1.21
Non-shrimp paste (*bagoong*)-eater		
At most 1x/month	0.44	0.23, 0.84
1x/week – 2x/day	0.99	0.51, 1.92

[a]*CYP1A1 g.6235T>C and NAT1*10 were removed from the model after backward logistic regression. Model Parameters: N = 487; -2LL = 447.045; R^2 = 0.31. Significantly different from previous model: X^2 = 9.90, df 4, p<0.05*

Discussion

The environmental factors shown by multivariate analysis to be significantly associated with oral cavity cancer in our study: smoking (current, passive and inverted); and tobacco chewing (Table 2), are known to be strongly associated with oral cavity cancer in literature.[1,32,33] On the other hand, environmental factors that were shown to be protective against oral cavity cancer among Filipinos—consumption of shrimp paste (*bagoong*) and fish sauce (*patis*)—have not yet been noted in previous studies. It is possible that fish sauce can serve as a source of polyunsaturated fatty acids (PUFAs), eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) which have been demonstrated in a number of in vitro and animal experiments to inhibit the promotion and progression of cancer; however, our findings merit further investigation since fish sauce in the diet has also been found to increase risk for esophageal and gastric cancer in previous studies.[34-38]

From the GST alleles included in this study, only the *GSTP1* polymorphism was found to be independently associated with oral cavity cancer among Filipinos. This polymorphism results in an Isoleucine to Valine substitution at position 105—close to the enzyme's binding site for electrophilic substrates.[39] Unsurprisingly, the 105Val variant of the enzyme has been demonstrated to exhibit altered affinity for electrophilic substrates.[40] Apart from causing substrate dependent changes in enzyme activity, this polymorphism was also shown to be less stable than the 105Ile form and has been associated with higher levels of DNA adducts.[17,41] Similar to the findings of this study, this *GSTP1* polymorphism has been noted to be a risk factor for this particular cancer type in a meta-analysis and previous studies among Taiwanese and Caucasian subjects.[6,42,43,44] It must be noted, however, that other genetic epidemiological studies conducted among Brazilians, Caucasians and one pooled analysis have found no association with *GSTP1* genotype and oral cavity cancer risk. With regard to the null variants of *GSTT1* and *GSTM1*, studies have demonstrated that homozygous deletion of either gene results in no functional activity of their respective enzyme.[6,45-48] Since both enzymes are involved in phase II detoxification carcinogens present in tobacco smoke, pesticides and other environmental pollutants functional inactivity for either *GSTM1* or *GSTT1* could result in reduced carcinogen detoxification and excretion and a higher rate of DNA-adduct formation, which could result in a higher risk for carcinogenesis.[16] The deletion at the *GSTM1* locus has been shown to be a significant risk factor in a number of studies among Japanese, Indian, Thai, and Asians.[49-53] However, this study was unable to detect any significant associations between the *GSTM1* and *GSTT1* null genotypes and oral cavity cancer risk among Filipinos. Concurrently, many other molecular epidemiology studies have reported a lack of genotype-risk associations for homozygous *GSTM1* deletions among Indian, Japanese, Korean, Taiwanese and Indonesian subjects, while a number of studies on the *GSTT1*null genotype have also reported no significant association with this cancer type among Indian, Japanese, Thai, Taiwanese, and Indonesian populations.[8,24,50,52,54-60] However a recent meta-analysis suggested that the GSTT1 null genotype is a risk allele for oral cancer among Asians, but more studies are needed to confirm this finding.[61]

Correlating the *CYP1A1* alleles and oral cavity cancer, published reports based on Indian, Japanese and Caucasian populations have identified the *CYP1A1 m2* allele or *m2*/*m2* genotype as a cancer risk factor; while the *CYP1A1m1*/*m1* genotype, which has been identified in studies among Korean and Japanese subjects and in one meta-analysis to significantly increase risk for oral cancer, has been shown to be a significant protective factor in this study.[8,24,49,58,62,63] One possible explanation for this discrepancy is that, among Filipinos, this allele is in linkage disequilibrium with a protective allele rather than with the functional allele, thereby producing the said results. On the other hand, a number of reports have been unable to detect significant associations with the *m1* and the *m2* alleles.[45,51,57,60,64]

Among the NAT variants, the *NAT1*10* allele, has been reported to be a significant risk factor for oral squamous cell-carcinoma in a study conducted on a Japanese population.[22] This particular allele is caused by two substitutions (g.1095C>A and g.1088T>A) in the 3' untranslated region of the gene, and has been associated with rapid acetylation both in vitro and in vivo.[29,65] Our findings suggest that the presence of the allele as well as the *NAT1*10* heterozygote genotype increase oral cavity cancer susceptibility. Despite being noted to be risk factors for oral cavity cancer in previous reports, the *NAT2* slow acetylating alleles (*NAT2*5A, NAT2*5B, NAT2*6B, NAT2*7A)* were not found to be significant in our study.[23,24] It must be noted that one

limitation of our study is the select number of *NAT1* and *NAT2* alleles investigated in our population. For such genes exhibiting such high genetic variability, interrogating a larger number of polymorphisms will allow for a more accurate determination of acetylator status, and in turn make for better evaluation of risk associations.

Subgroup analyses with respect to *GSTP1* c.313A>G, *CYP1A1* g.6235G>C and *NAT1*10* genotypes reveal that the effect of genotype combinations also reflect the oral cancer risk associations of the genetic factors when evaluated individually. When subjects were grouped according to *NAT1*10* status, susceptibility for oral cavity cancer among homozygotes for both *NAT1*10* and *GSTP1* c.313A>G risk alleles increased almost nine fold. This increased disease susceptibility that is associated with combining risk genotypes implies a synergistic relationship among different gene variants. After stratifying according to *CYP1A1 m1* allele status, the risk conferred by the homozygous *GSTP1* c.313A>G genotype was more than threefold higher among subjects who lacked or possessed only one copy of the *CYP1A1m1* allele—which was found to be protective in this study—whereas among subjects who had the CYP1A1 *m1/m1* genotype, the effect of *GSTP1 c.313A>G* was not significant.

After multivariate analysis of significant genetic and non-genetic variables, the *CYP1A1 m1* and *NAT1*10* genotypes were no longer significantly associated with oral cavity cancer, suggesting that the risk modifying effect of the environmental factors—smoking, passive smoking, inverted smoking, tobacco chewing, *patis* and *bagoong* consumption—were stronger relative to the effects of the *CYP1A1* and *NAT1* genes. The only gene variant that remained to be a significant risk factor for this cancer type was the homozygous *GSTP1* c.313A>G genotype. Our findings suggest that this polymorphism may play a significant role in the genesis of oral cavity carcinomas and it would be useful to explore its association with environmental factors may be modulated by this genetic polymorphism; moreover *GSTP1* is a good candidate as a risk modifier for oral cavity carcinoma since it is widely expressed in the oral cavity.[66] As for the other genetic polymorphisms which were not significantly associated with oral cavity cancer, the lack of association detected by our study does not automatically discount the potential of these genetic factors as risk modifiers for this cancer type. A genetic epidemiological study having a much larger sample size and greater allele coverage may be needed to better assess the relationship of these genes with disease susceptibility. To our knowledge, this is the first study that investigates the genetic epidemiology of oral cavity cancer in the Filipino population. The data obtained from this study will certainly serve as a useful reference for further studies to be conducted on existing and future hypotheses regarding relationships and risk modifying effects of the genetic and environmental variables studied.

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