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CASE–CONTROL STUDY OF ORAL AND OROPHARYNGEAL CANCER IN WHITES AND GENETIC VARIATION IN EIGHT METABOLIC ENZYMES

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Abstract

Background—Genetic variation in xenobiotic metabolizing enzymes may explain differing susceptibilities to the cancer causing effects of tobacco and alcohol.

Methods—We compared 203 oral squamous cell carcinoma cases and 416 controls for single nucleotide polymorphisms (SNPs) in 8 genes (*CYP1A1*, *CYP2E1*, *MPO*, *mEH*, *GSTM1*, *GSTT1*, *GSTP1*, and *NAT2*). Except for *NAT2*, genotype frequencies were similar in the 2 groups. We classified subjects as fast or slow NAT2 acetylators genotyping 13 *NAT2* SNPs.

Results—Fast acetylators were overrepresented in cases (53.7%) compared with controls (43.9%; odds ratio (OR) 1.55, 95% confidence interval (CI) 1.08–2.20; p value = .03). Gene–gene interaction testing suggested several cancer-NAT2 associations, with association strongest among persons without a *CYP1A1* variant (**2C* or **4*) allele (OR 1.77, 95% CI 1.20–2.60, p value = .03) or with a variant *MPO* (463A) allele (OR 2.38, 95% CI 1.34–4.21, p value = .05).

Conclusion—These results implicate fast NAT2 acetylation as a risk factor for oral cancer.

Keywords

tobacco; oral cancer; polymorphism; metabolizing enzymes; susceptibility

The American Cancer Society predicted 34,360 neworal (oral cavity and pharynx) cancer cases and 7550 deaths in 2007 among United States residents.[1] Oral cancer is the eighthmost frequent cancer worldwide, with striking variations in incidence according to geographic location.[2] Etiological factors contributing to variations in risk include cigarette smoking, oral tobacco, chronic alcohol use, and possibly human papillomavirus infection.[3] Despite tobacco and alcohol being such strong risk factors, few exposed individuals develop oral cancer.[4] Genetically determined interindividual differences in the metabolic ability to activate or eliminate tobacco or alcohol-associated carcinogens may determine personal

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susceptibility and may account, at least in part, for familial aggregation of oral cancer cases. Genetic polymorphisms have been described for Phase I and II metabolizing enzymes, and have been linked to phenotypic differences in enzyme activity or expression. Here, we report results from a study that compared 203 oral cancer cases and 416 control subjects for genetic differences in 8 genes involved in the metabolism of tobacco carcinogens or alcohol. Relevant background for the genetic endpoints selected for analysis is described below.

The cytochrome P450-dependent monooxygenases in particular, *CYP1A1* and *CYP2E1*, metabolize tobacco-associated carcinogens to activated intermediates.[5,6] A polymorphism at codon 462 in exon 7 of the *CYP1A1* gene has been associated with conferring increased susceptibility to oral cancer in whites,[7] whereas *CYP2E1* contains 2 linked polymorphisms[8] and studies in relation to tobacco-related cancer risk have been inconclusive so far.[9] A common G to A transition at position −463, in the myeloperoxidase (*MPO*) gene, has been associated with reduced *MPO* mRNA expression,[10] however, there are no reports on the association between the polymorphism and oral cancer risk.

Expressed in oral tissues, the human *mEH* gene contains 2 polymorphisms (Tyr113His and His139Arg) associated with altered mEH activity and have recently been associated with laryngeal, oral, and pharyngeal cancer risk.[11] Glutathione S-transferases (GSTs) are a supergene family coding for 5 multigene enzyme groups[12] that conjugate glutathione to easily excretable genotoxic electrophiles. The *GSTM1* null (0/0) polymorphism, the most widely studied, results in loss of protein expression and lack of association between the *GSTM1* null genotype and head and neck cancer has been reported.[13–17] However, a weak association was seen in a recent study in oral cancer patients.[18] Carriers of homozygous deletions of the *GSTT1* gene lack GST-θ activity in vivo.[19] While a meta-analysis of 21 studies showed a borderline risk for *GSTT1* null genotype and risk for squamous cell cancer of the head and neck,[20] Cheng et al reported a moderately strong association for oral cancer risk.[21] The *GSTP1* codon 105 variant has been associated with altered enzyme activity in vitro[22] and has been implicated as a susceptibility factor for oral cancer in 4 independent studies.[23–26]

Two polymorphic arylamine N-acetyltransferases enzymes (NAT1 and NAT2) catalyze the N-, O- or N,O-acetylation of the aryl- and heterocyclic amines. A slow acetylator phenotype, present in 40% to 70% of whites, has been associated with increased laryngeal cancer risk.[27] As reviewed by Ho et al, *NAT2* slow acetylator genotypes have been associated with mildly increased head and neck cancer risk, though not specifically with oral cancer risk.[28]

All SNPs reported to date are found within the 870-bp coding region of the *NAT2* gene. Eleven produce an amino acid substitution with 4 leading to decreased acetylation (single base-pair substitutions at positions 191, 341, 590, 857). Using the currently known human *NAT2* SNPs, we can now recognize specific *NAT2* haplotypes that are associated with acetylator function.[29,30] For example, the presence of SNP variants at positions 191, 341, 590, and 857 of the same chromosome identifies a defective *NAT2* product that contributes to a slow acetylator phenotype. Three distinct NAT2 phenotypes (slow, intermediate, and fast acetylator) can now be inferred based on the presence of homozygous, presence of heterozygous, and absence, respectively, of *NAT2* haplotypes associated with defective function. Earlier studies that were unable to make any distinction between the fast and intermediate acetylators only screened for 3 polymorphisms (C481T, G590A, and G857A). The validity of this approach assumes that no single chromosome could possibly have more than 1 variant SNP. Because genetic recombination occasionally disrupts the typical linkage pattern, this latter approach may misclassify subjects according to NAT2 status. To avoid these problems, we used a protocol that detects 13 relevant *NAT2* SNP variants and improves prediction of NAT2 phenotype.

Many published and unpublished studies have examined the association between genetically determined susceptibilities to environmental carcinogens and lung and head and neck cancer risk in adequately large sample sizes. However, there are no large studies of a single white population involving the simultaneous evaluation of the effects of several Phase I and II enzyme polymorphisms on oral cancer risk. The present study addresses this question by evaluating *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1*, *MPO*, *mEH*, and *NAT2* genetic variation in relation to oral cancer in individuals with some exposure to tobacco or alcohol.

PATIENTS AND METHODS

Study Population

Case Subjects—Beginning in February 2000, we sought referrals from oncology subspecialty ear, nose, and throat (ENT) surgeons operating at the University of Pittsburgh Medical Center (UPMC). Our request generally included patients with a recent diagnosis of primary squamous cell carcinoma of the head and neck. Eligible patients provided written informed consent, risk factor information through interviewer-administered questionnaire, and blood for genetic analysis.

We restricted analysis to patients enrolled between February 2000 and April 2004 who fit the following criteria: (1) biopsy-verified primary squamous cell carcinoma of the lip, oral cavity (mouth and anterior tongue), or oropharynx (soft palate, base of tongue, or tonsillar fossa); (2) study enrollment within 1 year of diagnosis; (3) 18 to 79 years old when diagnosed with a qualifying cancer; (4) white race; and (5) history of any cigarette or alcohol use. An exposure history required an affirmative response to at least 1 of 2 relevant questionnaire items (“Did you ever smoke at least 1 cigarette a day for 6 months or longer?” “Did you ever have 1 or more drinks per month for 1 year or longer?”).

We formally evaluated 677 referrals from ENT physicians and excluded (1) 242 (35.7%) not within 1 year of the diagnosis of a primary squamous cell carcinoma of the lip, oral cavity, or oropharynx; (2) 42 (6.2%) not between 18 and 79 years of age; (3) 19 (2.8%) nonwhite race; (4) 25 (3.7%) without cigarette or alcohol exposure history; and (5) 146 (21.6%) for other reasons (including unable to reach patient, lack of consent, unable to draw blood, lost blood sample, distant place of residence, and missing genotype information). The final case series included 203 patients, 6, 111, and 86 with lip, oral cavity, and oropharyngeal squamous cell cancer, respectively, 159 (78.3%) men, and mean enrollment age 58.7 years (range, 23–81 years; standard deviation, 10.8 years). ENT surgeons based at the university medical center and the affiliated Veterans Affairs Medical Center (VAMC) provided 196 and 7 case subjects, respectively.

Control Subjects—Between July 2000 and October 2002, we used electronic lists of registered voters and automobile drivers to sample potential control subjects who matched cases subjects according to sex, age (in 10-year strata), and residential zip code. We used mailed invitations with telephone follow-up to solicit participation in a genetic epidemiologic study of oral cancer and home visits to obtain informed consent, interview data, and blood samples from eligible persons.

Because of the expense and poor response rate associated with this community-based recruitment effort, we supplemented the control series with patients visiting the University of Pittsburgh Dental School outpatient service for routine dental care (recruitment starting in January 2001) and with patients visiting the VAMC for outpatient care (recruitment starting in April 2002). Field workers selectively approached and enrolled eligible subjects in a manner designed to improve gender, race, and age balance with the evolving case series.

We restricted analysis to control persons, enrolled before April 2004, who fit the following criteria: (1) no history (and, for dental school controls, no physical evidence) of head and neck cancer; (2) 18 to 84 years old when interviewed; (3) white race; and (4) history of any cigarette or alcohol use (as defined above). The final control series include 416 persons, 99, 237, and 80 from community, dental school, and VAMC sources, respectively, 302 (72.6%) men, and mean enrollment age 58.7 years (range, 27–84 years; standard deviation, 12.2 years).

Questionnaire Data

Using a questionnaire to obtain risk factor information, interviewers defined a reference date (1 year before cancer diagnosis for case subjects and 1 year before study selection date for control subjects) and framed questions to refer to the time period preceding the reference date. Variables used in analysis included sex, age on reference date, education, marital status, body mass index (weight in kg/height in meters squared), personal history of cancer (other than head and neck cancer), first-degree family history of cancer, and history of cigar, pipe, or smokeless tobacco use.

Each subject with a history of any cigarette use reported the average number of cigarettes smoked daily during discrete age periods over a life history that preceded the reference date. Accounting for gaps in smoking and changes in dose intensity, we produced a subject-specific cumulative cigarette exposure index (in pack-years) by summing (over age periods) the products of time duration (in years) and number of cigarettes smoked daily and dividing the result by 20 (the typical number of cigarettes in a pack of cigarettes).

Again, during discrete age periods over a life history that preceded the reference date, each subject with a history of any alcohol use reported the average number of days in a week or month he/she would drink and, on such days, the usual number of drinks consumed. We produced a subject-specific cumulative alcohol exposure index (in total drinks) by summing (over age periods) the products of time duration (in years), frequency of consumption (days per year), and usual amount consumed (drinks per day). Ex-smokers/exdrinkers were defined as those individuals who have stopped smoking or drinking for 1 year or more.

Genotyping Assays

We used commercial kits to isolate DNA from whole blood. *CYP1A1*2C* and *CYP1A1*4* polymorphisms were identified as BsrD1 and BsaI polymerase chain reactionrestriction-length-fragment polymorphisms (PCR-RLFP),[31,32] the *CYP2E1*5B* polymorphism as a RsaI PCR-RLFP of a 410 bp *CYP2E1* transcriptional regulation region PCR product,[33] the G to A *MPO* promoter region (position −463) polymorphism as a AciI PCR-RLFP,[10] Ala114Val and Ile105Val *GSTP1* polymorphisms as Alw261 and BsmAI PCR-RFLPs, respectively,[34] and the *mEH* Tyr113His (exon 3) and His139Arg (exon 4) polymorphisms as AspI and RsaI PCR-RLFPs, respectively.[35] Homozygous deletions of *GSTM1* and *GSTT1* were detected by means of differential PCR, with β-globin serving as internal control.[20,36] Finally, we used a Nanogen NanoChip Molecular Biology Workstation to distinguish 13 *NAT2* variants (111T>C, 190C>T, 191G>A, 282C>T, 341T>C, 411A>T, 481C>T, 590G>A, 759C>T, 803A>G, 845A>C, 857G>A, 859T>C).[29] Using the NAT2 allele nomenclature information as a guide, NAT2 haplotypes were constructed based on the partition ligation expectation maximization algorithm (PL-EM).[37] The haplotype pair with the greatest probabilitywas considered to be the haplotype phase for each individual. Laboratory qualitycontrol procedures included independent interpretation by 2 laboratory workers and 10% sample replicates. All replicate data showed 100% concordance with the original data.

Statistical Analysis

In the control group, we used the chi-square goodness-of-fit test and the exact test of Guo and Thompson[38] (implemented in SAS Genetics PROC ALLELE) to assess Hardy–Weinberg equilibrium for each of 21 SNPs in *CYP1A1* (2 SNPs), *CYP2E1* (1 SNP), *mEH* (2 SNPs), *MPO* (1 SNP), *GSTP1* (2 SNPs), and *NAT2* (13 SNPs) and a likelihood ratio test (SAS Genetics PROC HAPLOTYPE) to assess allelic association between each of the possible gene specific pairs possible.[39] To avoid multiple testing concerns, in a hypothesis driven fashion, we used the chi-square and Wilcoxon tests to determine the statistical significance (evaluated at $p < .05$, two-sided) of case–control differences with respect to categorical (including prospectively defined genotype classes) and continuous variables, respectively. In addition, we used the trend, allele case–control, and genotype case–control test statistics[40] (SAS Genetics PROC CASECONTROL, using 10,000 permutation samples and Monte Carlo methods to calculate exact *p* values) to evaluate possible association between disease status and each of 8 biallelic SNPs in *CYP1A1*, *CYP2E1*, *mEH*, *MPO*, or *GSTP1*. Separately, for each of 3 genes (*CYP1A1*, *mEH*, and *GSTP1*) characterized at more than 1 SNP locus, we used a likelihood ratio test statistic (implemented in SAS Genetics PROC HAPLO-YPE) to assess haplotype association with disease status.

We used the odds ratios (OR; eg, odds of belonging to a specified genotype class for case subjects divided by the odds of belonging to the genotype class for control subjects) to approximate relative risk (eg, oral cancer risk for persons in a specified genotype class divided by oral cancer risk for persons not in the genotype class). With case–control status as the dependent variable, we used logistic regression (SAS for Windows, version 9.1.3, SAS Institute, Cary, NC) to estimate the odds ratio (and 95%confidence interval), both unadjusted and adjusted for potential confounding factors.

Tabulating case–control status according to 1 genetic endpoint and stratifying according to a second genetic endpoint or environmental exposure factor (such as, cigarette or alcohol dose index), we used the Breslow–Day test for heterogeneity of the odds ratio to screen for gene–gene and gene–environment interaction, respectively.[41] Adding the relevant interaction term to logistic regression models, we also used the log-likelihood ratio test to determine the statistical significance of interaction effects.

RESULTS

Men, older age, personal cancer history (but not family cancer history), current cigarette smoking, current alcohol use, and ever use of cigarettes and alcohol were more frequent among the 197 oral cancer cases than the 416 controls without oral cancer (Table 1). Among ever smokers and ever drinkers, respectively, lifetime cigarette smoking and alcohol dose exposures were much greater in the case group than in the control group (Table 2).

When assessed in the control group, 1 (*GSTP1* Ala114Val) of 21 SNPs formally evaluated appeared to violate (*p* value = .03) Hardy–Weinberg equilibrium. The *GSTP1* Ile105Val and Ala114Val SNPs were linked ($D' = 0.79$, *p* value <.0001). Each of the other tested 27 SNP pairs satisfied allelic independence (every *p* value >.05).

CYP1A1, *CYP2E1*, *GSTM1*, *GSTT1*, *mEH*, *MPO* and *GSTP1* genotypes were similar in the case and control groups (Table 3). The trend, allele case–control, and genotype case–control test statistics did not identify statistically significant association involving any single *CYP1A1*, *CYP2E1*, *mEH*, *MPO*, or *GSTP1* SNP and oral cancer (every *p* value >.05). Separate omnibus likelihood ratio tests did not identify statistically significant association between oral cancer and *CYP1A1*, *mEH*, or *GSTP1* haplotype (every *p* value >.05). Variant frequencies matched those reported in the literature, with the possible exception of the

GSTM1 and *GSTT1* null genotype frequencies, which were observed at the upper end of the range of frequencies previously described for whites. Statistical control for sex, age, personal cancer history, family cancer history, smoking, and alcohol exposure variables did not alter judgments regarding the lack of association between oral cancer and *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, *mEH*, *MPO*, or *GSTP1* genotype (results not shown).

NAT2 genotypes associated with fast NAT2 function were more frequent in the case group (53.8%) than in the control group (43.9%; *p* value = .03; Table 3). This case–control difference, observed in persons with any cigarette or alcohol exposure history, suggested 55% (age- and sex-adjusted OR 1.55, 95% CI 1.08–2.20) higher oral cancer risk in association with a fast NAT2 acetylator status. Adjustments for personal cancer history and family cancer history did not alter the strength of association between *NAT2* genotype class and oral cancer risk. However, additional adjustments for cigarette smoking and alcohol history resulted in a significant odds ratio (OR 1.76, 95%CI 1.21–2.55).

Investigating 40 gene–environment pairs involving 8 genetic endpoints (Table 3) and 5 measures of cigarette or alcohol use, we identified only 1 instance of possible gene–environment interaction. The association between oral cancer and *CYP1A1* genotype (any **2C* or **4* allele vs no **2C* or **4* alleles) differed statistically (*p* value = .03) according to 1 measure of cigarette use (never smokers: OR 1.22, 95% CI 0.43–3.47; smokers ≤26 pack-years: OR 0.26, 95% CI 0.08–0.91; smokers >26 pack-years: OR 1.46, 95% CI 0.74–2.87). In an analysis restricted to current or excigarette smokers, the association between oral cancer and cumulative cigarette dose exposure (>26 pack-years vs ≤26 pack-years) differed statistically (*p* value <.01) according to *CYP1A1* genotype (any **2C* or **4* allele: OR 10.7, 95% CI 2.8–40.6; no **2C* or **4* alleles: OR 1.93, 95% CI 1.25–2.99).

The search for gene–gene interaction entailed separate study of the 28 possible gene–gene pairs established by our 8 genetic endpoints (Table 3). Analyses identified 2 statistically significant instances of interaction. The association between oral cancer and NAT2 acetylator status (fast vs slow) different statistically (*p* value = .03) according to *CYP1A1* genotype (any **2C* or **4* allele: OR 0.61, 95% CI 0.24–1.53; no **2C* or **4* alleles: OR 1.77, 95% CI 1.20–2.60). The association between oral cancer and *GSTP1* genotype (no wild-type haplotypes vs at least 1 wild-type haplotype) differed statistically (*p* value = .04) according to *CYP2E1* genotype (any **5B* allele: OR 0.22, 95% CI 0.01–4.24; no **5B* alleles: OR 1.44, 95% CI 0.83–1.52). A third gene–gene pair almost reached statistical significance. The association between oral cancer and NAT acetylator status (fast vs slow) differed with *p* value .051 according to *MPO* genotype (any 463A allele: OR 2.38, 95%CI 1.34–4.21; no 463A alleles: OR 1.16, 95%CI 0.74–1.80).

Using the 4 SNPs usually used to infer acetylator status, 7.6% of 276 NAT2 fast acetylators and 58.8% of 311 NAT2 slow acetylators, according to the 13-SNP classification method, were reclassified as NAT2 slow and fast, respectively. Reclassifications were independent of case–control status (*p* value >.05). The 4-SNP method increased the percentages regarded as being NAT2 fast (77.7% and 73.2% in the case and control groups, respectively). The NAT2-fast-oral-cancer association was statistically significant for cases and controls classified according to the 13-SNP method (crude OR 1.49, 95% CI 1.05–2.11), but not according to the 4-SNP method (crude OR 1.27, 95% CI 0.85–1.92).

DISCUSSION

Comparing 203 cases and 416 controls for genetic variation relevant to the metabolism of tobacco-related carcinogens and alcohol, we observed an independent and statistically significant association between oral cancer and a class of *NAT2* genotypes signifying fast

acetylation (multiply adjusted OR 1.76, 95% CI 1.21–2.55). Case and control genotype frequencies were similar for 7 other metabolic enzyme genes, *CYP1A1*, *CYP2E1*, *GSTM1*, *GSTT1*, *GSTP1*, *MPO*, and *mEH* (Table 3). We are unaware of any prior single report of as many genetic variants in as large a population of white subjects with oral squamous cell carcinoma. As part of a funded oral cancer program, we excluded subjects with head and neck cancer at sites other than the oral cavity or oropharynx. This homogeneous case group, restricted to oral cancer, may have created conditions favorable to the identification of causally relevant genetic factors. We also restricted the case and control groups to persons who admitted smoking cigarettes or drinking alcohol. This unique feature recognized not only the rarity of combined never drinking never smoking in oral cancer case series, but also the presumed action of our genetic endpoints through tobacco and alcohol.

Studies of *NAT2* and nonlaryngeal head and neck cancer are uncommon.[42–46] Two hospital-based studies, 1 French (121 oral/pharyngeal cancers, 164 controls)[42] and 1 Japanese (62 oral cancers, 122 controls),[43] observed moderate risk increases (OR 1.7- to 2-fold) with *NAT2* slow acetylator genotypes. Though also reporting an association with *NAT2* slow acetylator genotype, a second hospital-based Japanese study (145 head and neck cancer cases, 164 controls) included mostly cases of pharyngeal ($N = 43$) or laryngeal cancer ($N = 69$), with the risk association most apparent in the laryngeal cancer subgroup.[44] *NAT2* genotype frequencies were nearly identical in oral cancer case ($N = 94$) and control groups ($N = 92$) from a German clinic-based study.[45] A U.S. population-based case–control study from the state of Washington (white subjects: 320 oral cancers, 520 controls), reported statistically nonsignificant inverse oral cancer risk associations with intermediate (OR 0.9, 95% CI 0.5–1.5) and slow (OR 0.9, 95%CI 0.5–1.7) *NAT2* genotype.[46]

Studies investigating the modifying effects of *NAT2* on tobacco or alcohol-related oral cancer risk aremore uncommon. The French study, mentioned above, observed a slow versus fast *NAT2*-cancer association (OR 3.1, 95% CI 1.3–7.2) only in persons with a smoking history ≤30 years in duration.[43] In the U.S. study, the alcohol-cancer association was stronger in nonslow (rapid or intermediate) than slowNAT2 acetylators.[46] However, we are unaware of any direct role for NAT2 in the metabolism of ethanol or acetaldehyde (a carcinogen produced by the action of aldehyde dehydrogenase on ethanol). Alcohol intake may alter the action of *NAT2* variants on oral cancer risk through induction of other enzymes involved in tobacco carcinogen activation-detoxification. We observed a stronger association between oral cancer and cumulative cigarette dose exposure among persons with at least 1 variant *CYP1A1* SNP (**2C* or **4*). However, we did not observe any similar interactions involving NAT acetylator status and measures of alcohol or cigarette use.

In contrast to this report of an association between NAT2 fast acetylator status and increased oral cancer risk, studies of smoking-related cancer at other sites, such as bladder, esophagus, larynx, and lung, often suggest association in the opposite direction. Depending on inhalational as opposed to noninhalational route of exposure, variable contact with distinct tobacco-related carcinogens, including polycyclic aromatic hydrocarbons and aromatic amines, may explain results that differ according to cancer site. The highly polymorphic N-acetyltransferases both activate and inactivate many different chemicals that belong to distinct chemical classes. Therefore, the direction of effects from *NAT2* differences and associated mechanisms of action may be difficult to predict for complex carcinogen mixtures. In addition, we classified cases and controls according to 13 *NAT2* alleles, as opposed to the 4 or 5 most common alleles typically studied. As noted above, the 4-SNP and 13-SNP approaches classified subjects differently according to NAT2 acetylator status. In addition to possible interactions with ethnic mix, cigarette type, dose intensity, other smoking behaviors (eg, depth of inhalation), alcohol, or dietary intake, choice of genetic endpoint may contribute to variable results across studies or across cancer sites.

Limitations of our study include restriction to a single (white) racial group and sample size, though larger than most other studies of oral cancer. We have not adjusted for HPV16 status, 1 of the major risk factors for pharyngeal cancer, however the genotype frequencies were statistically similar in oral cases versus oropharyngeal cases within this case group. As determinants of oral cancer risk, metabolic enzymes presumably act as members of pathways or networks. We observed 3 situations where genetic variation involving a Phase I enzyme affected the association between oral cancer and genetic variation involving a Phase II enzyme (*CYP1A1* and *NAT2*, *CYP2E1* and *GSTP1*, and *MPO* and *NAT2*). However, confident study of gene-gene interactions of this nature requires much larger sample sizes. Finally, when examining a set of candidate genes, human geneticists debate the advantages and disadvantages of approaches based on study of phased haplotype, as opposed to genotype. Our approach stressed genetic risk factor classification schemes consistent with a current understanding of the biological properties of the genetic variants selected for study. Particularly for *NAT2*, a gene showing extensive genetic variability in human populations, analyses based on empirically derived haplotypes, as opposed to genotype classes defined to represent biological differences, may or may not lead to similar conclusions. The tools available for the genetic dissection of complex traits have matured and whole genome association studies might provide new insights in the future.

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Table 1

Characteristics of cancer case and noncancer control subjects.

	Cases (*N* = 197)		Controls (*N* = 416)		
Risk factor	**No.**	**%**	**No.**	**%**	**Chi-square *p* value**
Sex, male	154	78.2	302	72.6	.14
Reference age					.003
<50 y	38	19.3	123	29.6	
50–59 y	79	40.1	111	26.7	
60–69 y	47	23.9	100	24.0	
70+ y	33	16.8	82	19.7	
Personal cancer history	28	14.2	44	10.6	.20
Family cancer history	117	59.7	239	57.7	.65
Cigarette use					<.0001
Never smoker	39	19.9	106	25.5	
Ex-smoker	63	32.1	186	44.8	
Current smoker	94	48.0	123	29.6	
Alcohol use					.0008
Never drinker	6	3.0	44	10.6	
Ex-drinker	49	24.9	126	30.3	
Current drinker	142	72.1	246	59.1	
Cigarette by alcohol use					.0007
Ever smoker–never drinker	6	3.0	44	10.6	
Never smoker–ever drinker	39	19.8	106	25.5	
Ever smoker–ever drinker	152	77.2	265	63.9	

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Table 2

Lifetime cigarette smoking and alcohol use dose in cancer case and noncancer control subjects.

Risk factor	Cases	Controls	*p* value*
Cigarette index (pack-years)†,‡	(*N* = 158)	(*N* = 307)	<.0001
Median	38	26	
Interquartile range	22–62	10–42	
Range	<1–188	<1–147	
Alcohol index (cumulative drinks)†,§	(*N* = 191)	(*N* = 367)	<.0001
Median	15,808	5,928	
Interquartile range	3,200–54,600	1,400–21,500	
Range	<100–393,100	<100–273,000	

*Wilcoxon rank-sum test.

†Among persons with any history of use.

‡Cigarette index could not be calculated for 2 control subjects with a history of ever smoking cigarettes.

§Alcohol index could not be calculated 5 control subjects with a history of ever drinking alcohol.

Table 3

Genotyping results in cancer case and noncancer control subjects.

	Case (*N* = 197)		Control (*N* = 416)		
Genotype	**No.**	**%**	**No.**	**%**	***p* value †**
CYP1A1					.73
Not done	7	(3.4)	6	(1.4)	
*1/*1	162	85.3	345	84.1	
non-*1/*1	28	14.7	65	15.9	
CYP2E1					.36
Not done	7	(3.4)	13	(3.1)	
G/G C/C	176	92.6	364	90.3	
non-G/C C/C	14	7.4	39	9.7	
GSTM1					.5
Not done	1	(0.5)	2	(0.5)	
Null	126	64.3	276	66.7	
non-null	70	35.7	138	33.3	
GSTT1					.22
Not done	2	(1.0)	2	(0.5)	
Null	67	34.4	122	29.5	
non-null	128	65.6	292	70.5	
mEH activity					.95
Not done	1	(0.5)	2	(0.5)	
rapid	34	17.3	70	16.9	
normal	86	43.9	192	46.4	
slow	62	31.6	126	30.4	
very slow	14	7.1	26	6.3	
MPOG463A					.99
Not done	0	(0.0)	2	(0.5)	
G/G	123	62.4	259	62.6	
G/A	64	32.5	133	32.1	
A/A	10	5.1	22	5.3	

Genotype	Case (*N* = 197) No.	%	Control (*N* = 416) No.	%	*p* value †
GSTP1					.74
Not done	1	(0.5)	2	(0.5)	
A/A, A/B, A/D	155	79.1	336	81.2	
B/B, B/C, C/C, C/D, D/D	24	12.2	42	10.1	
A/C or B/D	17	8.7	36	8.7	
NAT2					.03
Not done	15	(7.4)	17	(4.1)	
Fast	98	53.8	175	43.9	
Slow	84	46.2	224	56.1	

† Chi-square, genotype distribution in case versus control subjects, excluding subjects with missing genotype.