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Polymorphisms in *NAT2* and *GSTP1* Are Associated With Survival in Oral and Oropharyngeal Cancer

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Abstract

Introduction—Functional polymorphisms in drug metabolizing enzymes (DMEs) may be determinants of survival in oral and oropharyngeal squamous cell carcinoma (OOSCC).

Methods—OOSCC cases (N=159) with a history of either tobacco or alcohol use were genotyped for polymorphisms in eight DMEs. Overall and disease-specific survival were analyzed using Kaplan-Meier plots and the log-rank test. Cox proportional hazards regression was used to calculate hazard ratios (HR) and 95% confidence intervals (CI) in exploratory analyses of patient subgroups.

Results—Kaplan-Meier analyses showed N-acteyltransferase-2 (*NAT2*) fast acetylators experienced a 19.7% higher 5-year survival rate than slow acetylators (*P*=0.03) and this association was similar in oropharyngeal and oral cancer. After multiple adjustment, including tumor site and stage, the *NAT2* fast acetylator phenotype was associated with improved overall survival (vs. slow acetylators) provided chemotherapy or radiation were not used (HR, 0.26; 95% CI, 0.10–0.66). However, *NAT2* phenotype was unrelated to survival in patients treated with chemoradiotherapy (HR, 1.21; 95% CI, 0. 54–2.73) or radiotherapy (HR, 0.67; 95% CI, 0.31–1.59) (*P*-for-*NAT2*/treatment-interaction=0.04). Normal activity *GSTP1* was associated with a

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Conflict of Interest Statement
None of the authors have any conflict of interest to declare.

19.2% reduction in 5-year disease-specific survival relative to reduced activity *GSTP1* (*P*=0.04) but this association was not modified by treatment.

Conclusions—Our results suggest that functional polymorphisms in *NAT2* and *GSTP1* are associated with OOSCC survival. Confirmation of these results in larger studies is required.

Keywords

head and neck neoplasms; NAT2; GSTP1; polymorphism single nucelotide; SNP

Introduction

Oral and oropharyngeal squamous cell carcinomas (OOSCC) are the 10th most common cancer and 7th most common cause of cancer death worldwide.[1] The majority of OOSCC are associated with tobacco and alcohol use, and risk associated with these behaviors is modified by functional polymorphisms in genes encoding drug metabolizing enzymes (DMEs).[2–7] DMEs are relevant in cancer survival due to their role in metabolism of cancer chemotherapies[8] and commonly encountered dietary and environmental carcinogens.[9, 10] In addition, 20–40% of OOSCC patients continue smoking after diagnosis,[11–14] implying tobacco-metabolic DMEs may affect survival in a substantial proportion of OOSCC patients. The study of DMEs and OOSCC survival is of particular interest considering advances in OOSCC treatment have not substantially impacted survival. [15, 16] However, few studies of DMEs and OOSC survival are available. The *CYP1A2*1C* polymorphism has been associated with reduced disease-free survival,[17] non-null *GSTT1* was associated with reduced overall survival,[18] and non-null *GSTM1* was associated with increased risk of second primary tumors.[19]

Given the paucity of data on OOSCC survival associated with DMEs, we conducted a preliminary investigation of overall and disease-specific survival associated with polymorphisms in eight DMEs associated with metabolism of tobacco, alcohol, chemotherapies, and dietary/environmental toxins: *mEH, MPO, CYP1A1, CYP2E1, NAT2, GSTT1, GSTM1,* and *GSTP1*. All cases included in our study completed an interviewer-administered questionnaire soliciting tobacco/alcohol use, anthropometry, diet, and oral care habits,[20] allowing us to explore gene/environment interactions with the objective of identifying hypotheses for investigation in future research studies.

Methods

Patients

The case series for this analysis has been described previously.[20] OOSCC cases (*N*=203) were recruited at University of Pittsburgh Medical Center otolaryngology clinics during 2000–2004 for participation in a case-control study of OOSCC etiology, including polymorphisms in DMEs.[20] Cases were age 18–79 at diagnosis with biopsy-verified primary lip, oral cavity (mouth or anterior tongue) or oropharyngeal (base of tongue, tonsil fossa, or soft palate) squamous cell carcinoma within 1 year of interview (excluding *in-situ* cancer), white race only, and were self-reported smokers or drinkers (smoked >= 1 cigarette per day for >= 6 months or consumed >= 1 drink/month for >= 1 year).

For our analysis, we required oral and oropharyngeal cases only, treated at our institution for their first-ever OOSCC, and who consented to follow-up. Therefore, we excluded 44 (22%) of the original 203 cases: 6 lip cancers, 5 cases later found ineligible for the original study (3 with *in situ* and 2 with recurrent disease), 22 cases who did not consent to follow-up, 4 cases not treated at our institution, 3 cases with undocumented tumor site, 1 case with unknown diagnosis date, and 3 cases treated at our institution for a second primary tumor or

recurrence. This left 159 cases (92 oral cavity and 67 oropharyngeal) for analysis. Excluded cases were more likely to be underweight (22.7%) than included cases (2.5%) (*P*<0.001).

All cases consented to the use of their genotype, questionnaire, and follow-up information. This study was approved by the University of Pittsburgh Institutional Review Board.

Genes Analyzed and Genotype Assays

The genes we analyzed, including their association with OOSCC and details of genotyping procedures used in our case series, have been described previously.[20] Briefly, our analysis focused on polymorphisms in eight genes associated with survival in cancers other than OOSCC (*mEH*[21], *MPO*[22], *NAT2*[23],[24]), or genes with biologically plausible associations with OOSCC survival through metabolism of polycyclic aromatic hydrocarbons (PAHs) or ethanol (*mEH*[7], *MPO*[25], *NAT2*[9], *CYP1A1* and *CYP2E1*[4, 26]), or OOSCC chemotherapies (*GSTM1*, *GSTT1*, and *GSTP1*[8, 27, 28]).

Polymorphisms in *mEH, MPO, CYP1A1, CYP2E1*, and *GSTP1* were identified by polymerase chain reaction (PCR) and restriction fragment length polymorphism; homozygous deletions of *GSTT1* and *GSTM1* were identified by differential PCR; and *NAT2* phenotype was predicted using international consensus criteria after genotyping thirteen SNPs using a Nanogen NanoChip Molecular Biology Workstation and algorithmic gametic phasing check.[20, 29]

Survival Endpoints and Outcome Ascertainment

We designated 5-year survival as a clinically relevant primary endpoint. Overall survival time was calculated from the procedure date (the date of primary treatment [surgery or first radio- or chemoradiotherapy]) to the date of death from any cause. Disease-specific survival time was calculated from the procedure date to the date of death from OOSCC. Deaths were ascertained by monthly analysis of an electronic patient registry and verified using the Social Security Death Index. Cause of death was assigned using information recorded at the time of death or last contact prior to death. Cases were censored if they were not known to have died during the study period (all analyses) or if they died of causes other than OOSCC (disease-specific survival). We considered follow-up through December 31, 2010.

Exposure Variables

The following variables were of primary interest: CYP1A1 (wild type [*1/*1] vs. mutant), CYP2E1 (wild type [G/G, C/C] vs. mutant), mEH (slow, normal, and rapid), MPO463G>A (wild type [G/G] vs. mutant), GSTP1 (normal activity diplotype [*A/*A, *A/*B, *A/*D] vs. reduced activity diplotype [*A/*C, *B/*B,*B/*C, *B/*D, *C/*C, *C/*D, and *D/*D] where *A, *B, *C, and *D refer to conventional Ile105Val-Ala114Val haplotypes as follows: *A=Ile-Ala (wild type), *B=Val-Ala, *C=Val-Val, and *D=Ile-Val),[27] GSTT1 and GSTM1 (homozygous null vs. any non-null), and NAT2 (fast vs. slow acetylator). We also defined: sex, tumor stage (I/II, III/IV), age at diagnosis (continuous), tumor site (oral cavity or oropharynx), cigarette smoking (ever vs. never), alcohol drinking (ever vs. never), BMI [kg/m^2] 1 year before diagnosis (underweight [<18.5], normal [18.5–24.9], overweight [25.0–29.9], and obese [>=30]), personal history of cancer (yes/no), and cancer in a first-degree relative (yes/no). Treatment was available from medical records and was defined as radiotherapy (with or without surgery), chemoradiotherapy (with or without surgery), or no chemotherapy/radiotherapy. For use in exploratory analyses, we defined education (grade school, high school, vocational, or college), servings/day (continuous) of fruit and vegetables (separately), eating habits at interview unchanged compared with 3–5 years ago (yes/no), US vs. non-US birthplace, teeth brushing frequency (continuous; times/day).. For smokers, we defined: maximum number of cigarettes smoked/day (continuous), duration of

smoking (continuous), pack-years (continuous; product of maximum number of cigarettes/ day and duration), and years since quitting (continuous).

Statistical Analysis

In our primary analyses, associations between clinicopathological factors and survival were assessed using the Kaplan-Meier method and the log-rank test. We also selected genes with log-rank P-values <= 0.10 for further exploratory analyses using Cox proportional hazards regression. For each selected gene, the gene itself, as well as tumor site, stage, and treatment, were forced into the model. Other main effects were tested one at a time, with the final model including all significant (alpha=0.20) main effects. Continuous first-order interactions between the gene of interest and other predictors were tested one at a time. Tests for statistical significance were conducted using the likelihood ratio Chi square test. Tests for trend were conducted only among cases with the factor of interest by adding a continuous variable (symbolizing a 1-unit change) to the final model. All statistical tests based on the final model used a 2-sided alpha=0.05. Proportional hazards were verified graphically and no violations were observed.

Due to overlap in substrate specificity of glutathione S-transferases (GST),[30] we explored the joint impact of *GSTP1*, *GSTT1*, and *GSTM1* on disease-specific survival using Cox proportional hazards regression. First, we explored univariable associations between these genes and OOSCC death. Then, we summed the number of conjugation-reducing mutations per patient (i.e., *GSTM1*-null, *GSTT1*-null, and reduced activity *GSTP1*) and modeled this as a continuous predictor of OOSC death.

Analyses were performed using SAS 9.2 (SAS Institute, Cary, NC).

Results

The 159 cases included in this study (Table 1) were predominantly male (77.4%), between the ages of 50–69 (61.0%), stage III/IV (69.0%), and represented primarily oral cancer (57.9%). All cases were ever-smokers or ever-drinkers, with the majority (76.1%) reporting a history of both. A total of 95 (60.1%) cases had a first-degree relative with cancer while only 16 (10.1%) reported a personal cancer history. Treatments administered by tumor site and stage are shown in Table 2. Among cases receiving chemotherapy, 17 (29.8%) received a single platinum agent, 5 cases (8.8%) received platinum with 5-fluorouracil, 28 (49.1%) received platinum with a taxane, and chemotherapy was undocumented for 7 (12.3%) cases. Median follow-up was 5.3 years (range: 0.1–10.8). A total of 79 (49.7%) cases died, including 40 deaths from OOSCC.

Overall Survival

Older age (P=0.02), smoking/drinking (P=0.05), and higher stage (P=0.09) were associated with reduced overall survival (Table 1). Analysis of polymorphisms (Table 3) showed *NAT2* fast acetylators experienced a 19.7% higher 5-year survival rate than slow acetylators (P=0.03) and this association was similar in oropharyngeal and oral cancer (Figure 1). *NAT2* phenotype was similar in oral and oropharyngeal cases, and was unrelated to clinicopathological factors and other DME polymorphisms (data not shown). In our exploratory analyses, improved survival associated with the *NAT2* fast acetylator phenotype was no longer significant after multiple adjustment (HR, 0.64; 95% CI, 0.40–1.04) (Table 4). However, after controlling for tumor site and stage, we noted a statistically significant *NAT2*-treatment interaction (P=0.04). Specifically, we observed a survival benefit associated with the *NAT2* fast acetylator phenotype only among cases not receiving chemotherapy or radiation (HR, 0.26; 95% CI, 0.10–0.66). We did not observe this

association in patients treated with radiotherapy (HR, 0.67; 95% CI, 0.31–1.59) or chemoradiotherapy (HR, 1.21; 95% CI, 0.54–2.73). Caution is warranted in interpretation of this finding, however, given the small number of deaths among *NAT2* fast acetylators treated without chemotherapy or radiation. Finally, no trends in risk of death were observed for duration of smoking, cigarettes/day, pack-years, or years since quitting (*P*>0.05 for all). In addition, results were unchanged after adjustment for level of education, BMI, daily servings of fruit or vegetables, consistency of eating habits, US vs. non-US birthplace, number of times per day teeth were brushed, and personal or first-degree relative cancer history (*P*>0.10 for all). We did not observe any interaction between *NAT2* and age, sex, smoking status, and daily servings of fruit or vegetables (*P*>0.20 for all). Finally, polymorphisms in DMEs other than *NAT2* were not associated with overall survival (Table 3).

Disease-Specific Survival

Clinicopathological factors, except for late stage (*P*=0.04), were not strongly associated with disease-specific survival (Table 1). Normal activity *GSTP1* was associated with a 19.2% reduction in 5-year disease-specific survival (*P*=0.04) (Table 3) but *GSTP1* was not associated with OOSC death in our exploratory multivariable model and did not interact with treatment (Table 4). However, we did observe a *GSTP1*-sex interaction in which reduced-activity *GSTP1* was associated with an 88% reduction in risk of OOSCC death among men (HR, 0.12; 95% CI, 0.02–0.91) but not women (HR, 2.29; 95% CI, 0.41–12.69) (P-for-interaction=0.02) (Table 5). However, this result must be considered preliminary as hazard estimates among several combinations of *GSTP1* and sex were based on a small number of deaths (Table 5). Adjustment for demographic and lifestyle factors did not alter these results (data not shown). When considering the total number of GST conjugation-reducing polymorphisms in each patient, we noted each additional polymorphism was associated with a 35% reduction in risk of OOSCC death (HR, 0.65; 95% CI, 0.43–0.98). Results were unchanged after controlling for sex and treatment, and no interaction was observed with either factor (data not shown). Finally, no other DMEs studied were associated with disease-specific survival (Table 3).

Discussion

During 700 person-years of follow-up among 159 cases, we observed improved overall survival among *NAT2* fast acetylators and improved disease-specific survival associated with reduced activity *GSTP1*. Genotype of *NAT2* and *GSTP1* were unrelated to tumor site or any other clinicopathological factors in our case series. Results of our exploratory regression analyses suggested the *NAT2* survival benefit was strongest in cases not receiving chemotherapy or radiotherapy, where reduction in risk of death was 74% after adjustment for smoking history, tumor site, and tumor stage. The interaction of *NAT2* phenotype with treatment was significant even after control for tumor stage, suggesting aspects of advanced disease did not produce the pattern we observed.

There are at least sixty known *NAT2* polymorphisms grouped into "slow" and "fast" acetylator phenotypes that have been associated with cancer risk.[29, 31] However, we were unable to identify previous reports of *NAT2* polymorphisms and survival in OOSCC, and *NAT2* is not strongly associated with survival in other cancers.[23, 24, 32–35] Our positive finding for *NAT2* might be explained by an improved prediction of *NAT2* phenotype based on the use of thirteen SNPs.[20] The biological mechanism through which *NAT2* might affect survival is unclear.[36] *NAT2* is a Phase II enzyme expressed primarily in the liver and its substrates are commonly found in the environment, e.g., heterocyclic and aromatic amines in cigarette smoke, diesel exhaust, and roasted meat.[9] Therefore, our observation of improved survival among fast acetylators in the absence of chemotherapy or radiotherapy

may reflect an impact of *NAT2* on environmental exposures in patients unencumbered by treatments that otherwise overwhelm the benefits of fast acetylation. In addition, radiation and platinum chemotherapies are not substrates of *NAT2* and their impact on survival is not expected to be modified by *NAT2*. If *NAT2* modifies survival through metabolism of environmental toxins, it seems reasonable that the *NAT2*-survival association would become apparent only after prolonged exposure. Indeed, we observed survival curves did not separate until two years after cases underwent their first medical procedure. Examples of prolonged exposures might include dietary patterns and continued smoking. While we did not detect significant modification of the *NAT2*-survival association by fruit or vegetable consumption, our questionnaire did not directly measure the major dietary source of *NAT2* substrates--roasted meat.[9] Unfortunately, smoking status post-diagnosis was not recorded in our study, although this behavior is reportedly common, with 20%–40% of head and neck cancer patients continuing to smoke.[11–14]

Our Kaplan-Meier analysis also showed *GSTP1* was associated with disease-specific survival, but this association did not persist after multiple adjustment. *GSTP1* is a Phase II enzyme expressed throughout the body and is known to detoxify the platinum chemotherapies used in our case series.[30, 37] Previous research shows an association between reduced activity *GSTP1* and improved response to chemotherapy in head and neck cancer,[38] as well as improved survival in lung, colorectal, and ovarian cancers.[8] We are aware of only one prior report of *GSTP1* and overall or disease-specific survival that included OOSCC, and this report showed no association between reduced activity *GSTP1* and disease-specific survival among 190 oral, pharyngeal, and laryngeal cancer cases.[18] However, only 19% of cases in this study received chemotherapy.[18] Despite the more extensive use of chemotherapy in our study (35.8% of cases received chemotherapy and platinum agents were used extensively), we did not observe interaction between *GSTP1* and treatment. This may be attributable to small subgroup sizes defined by six combinations of *GSTP1* activity and treatment. In addition, our observation of progressively improving survival with decreasing ability to conjugate substrates to glutathione suggests that a comprehensive measure of GST function may be a more important predictor of survival than polymorphisms in any single GST alone. Finally, we observed that reduced activity *GSTP1* was associated with improved survival in men only. While we cannot ignore that our estimate of the *GSTP1*-sex interaction was based on a small number of deaths, differences in survival between sexes with the same *GSTP1* polymorphism seem plausible.[39] Larger case series will be required for future studies of this association.

Our results are accompanied by several limitations. First, if genetic variants of DMEs not included in our study are inherited with *NAT2* or *GSTP1* polymorphisms, this may confound our results through associations with *NAT2* or *GSTP1* and survival. In addition, measurement of *NAT2* alone may not adequately classify acetylator phenotype as this enzyme shares substrates with *NAT1*.[9] Furthermore, our method of vital status ascertainment may have resulted in failure to record deaths during the study period. However, this should not impact our results as cases were known to be alive when censored at last contact. In addition, we observed similar survival comparing oral and oropharyngeal cases. While oropharyngeal tumors are often associated with improved survival due to a more frequent HPV-related etiology, our results apply largely to smoking-related OOSCC as smokers were specifically selected for our study. Finally, our results are based on a relatively small sample and we estimate our study provided 60% power to detect the main effect of *NAT2* on overall survival that we observed (details not shown). In addition, substantial caution is warranted in interpretation of the results of our regression analyses given the small number of deaths in subgroups defined by the various interactions we studied.

In summary, we observed a benefit of *NAT2* fast acetylation on overall survival in OOSCC, which we believe has not been reported previously, and improved disease-specific survival associated with reduced activity *GSTP1*. Our results for *NAT2* may reflect interaction with lifestyle or other environmental exposures post-diagnosis and future studies of *NAT2* and survival in OOSCC should assess such factors. Larger case series will be required to investigate associations between *GSTs* and survival in OOSCC subgroups. Finally, confirmation of our results in larger studies may contribute to an understanding of the determinants of survival in OOSCC patients.

Acknowledgments

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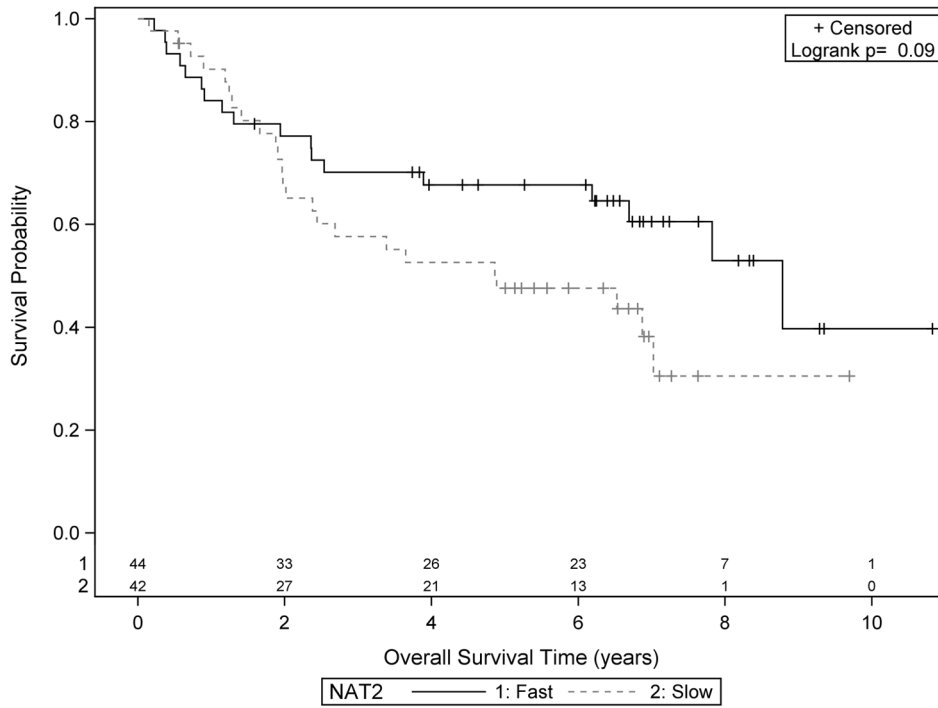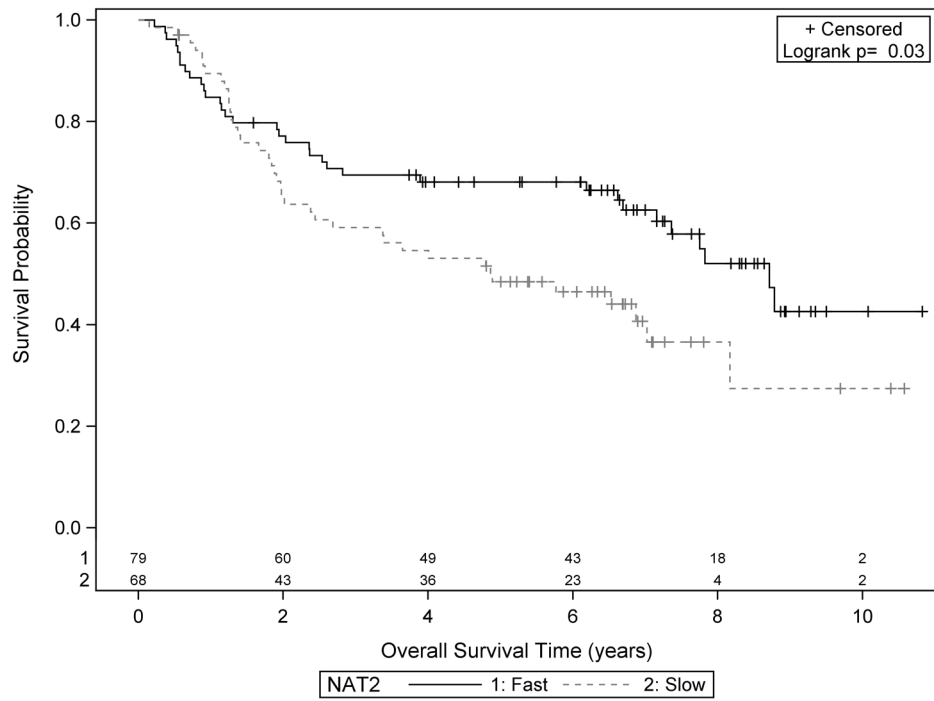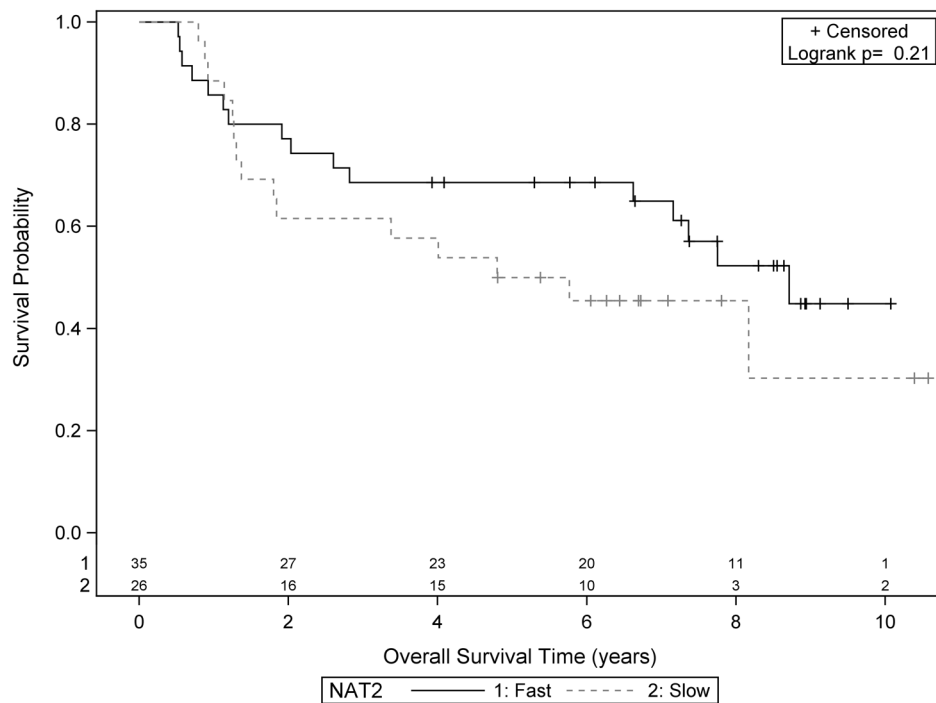
Figure 1.
Figure 1a. Kaplan-Meier plot of overall survival (including number at risk) associated with predicted *NAT2* phenotype among all cases.
Figure 1b. Kaplan-Meier plot of overall survival (including number at risk) associated with predicted *NAT2* phenotype among oral cancer cases only.
Figure 1c. Kaplan-Meier plot of overall survival (including number at risk) associated with predicted *NAT2* phenotype among oropharyngeal cancer cases only.

Table 1

Clinicopathological Characteristics in Relation to Outcome Among 159 Oral and Oropharyngeal Cancer Cases

	N	%	Overall Survival			Disease-Specific Survival		
			Deaths	% Surviving 5 Years[1]	P-value[2]	Deaths	% Surviving 5 Years[1]	P-value[2]
Age					0.02			0.59
< 50	39	24.5	15	66.2		8	79.5	
50–59	57	35.8	24	65.7		18	68.8	
60–69	40	25.2	22	57.5		11	71.2	
>=70	23	14.5	18	35.2		3	76.4	
Sex					0.19			0.15
Female	36	22.6	15	66.6		6	82.1	
Male	123	77.4	64	57.4		34	70.3	
Race					0.19			0.15
White	159	100.0	79	59.5		40	73.1	
Site					0.78			0.54
Oral cavity	92	57.9	44	58.3		21	74.6	
Oropharynx	67	42.1	35	61.1		19	70.9	
Stage[3]					0.09			0.04
Stage I/II	49	31.0	21	72.4		8	84.9	
Stage III/IV	109	69.0	58	53.4		32	67.3	
Smoking/Drinking					0.05			0.76
Ever drinker only	33	20.8	11	72.7		8	75.5	
Ever smoker only	5	3.1	2	80.0		1	80.0	
Ever drank & ever smoked	121	76.1	66	54.8		31	71.9	
BMI 1 year pre-diagnosis					0.75			0.70
<18.5	4	2.5	2	75.0		0	100.0	
18.5–24.9	67	42.1	37	50.3		18	70.4	
25–29.9	52	32.7	24	67.8		13	75.2	
>=30	36	22.6	16	63.0		9	71.8	

	N	%	Deaths	% Surviving 5 Years[1]	P-value[2]	Deaths	% Surviving 5 Years[1]	P-value[2]
			Overall Survival			**Disease-Specific Survival**		
First-degree Relative Had Cancer[4]					0.71			0.56
Yes	95	60.1	48	61.1		22	75.6	
No	63	39.9	31	56.3		18	68.9	
Personal History of Cancer[4]					0.22			0.40
Yes	16	10.1	10	43.8		5	64.3	
No	143	89.9	69	61.3		35	74.0	

[1] Kaplan-Meier survival estimate

[2] Log-rank test

[3] Stage is missing for 1 case

[4] Personal and first-degree relative cancer history is missing for 1 case

Table 2

Treatment of Oral and Oropharyngeal Cancer Cases (N=159)

A) Treatment By Tumor Site		Oral cavity	Oropharynx
Radiotherapy (w/ or w/out surgery)[1]	N (%)	20 (51.3)	19 (48.7)
Chemoradiotherapy (w/ or w/out surgery)[1]	N (%)	19 (33.3)	38 (66. 7)
No Chemotherapy/Radiotherapy	N (%)	53 (84.1)	10 (15.9)

B) Treatment By Tumor Stage		Stage 1/2	Stage 3/4	Unknown
Radiotherapy (w/ or w/out surgery)[1]	N (%)	8 (20.5)	31 (79.5)	
Chemoradiotherapy (w/ or w/out surgery)[1]	N (%)	2 (3.5)	54 (94.7)	1 (1.8)
No Chemotherapy/Radiotherapy	N (%)	39 (61.9)	24 (38.1)	

A total of 32 cases (82.1%) who received radiotherapy also received surgery, and 21 cases (36.8%) who received chemoradiotherapy also received surgery.

Table 3

Genotype and Outcome among 159 Oral and Oropharyngeal Cancer Cases

	N[1]	%	Deaths	Overall Survival			Deaths	Disease-Specific Survival	
				% Surviving 5 Years[2]	P-value[3]			% Surviving 5 Years[2]	P-value[3]
NAT2					0.03				0.06
Fast	79	53.7	34	68.1			14	80.6	
Slow	68	46.3	39	48.4			22	64.1	
GSTP1					0.14				0.04
Reduced Activity	28	17.7	11	75.0			3	88.7	
Normal Activity	130	82.3	67	56.5			37	69.5	
CYP1A1					0.74				0.67
*1/*1	125	82.2	62	62.5			31	74.2	
non-1/1	27	17.8	14	46.1			8	64.7	
CYP2E1					0.16				0.20
G/G C/C	140	92.1	71	59.2			36	72.8	
non-G/G C/C	12	7.9	3	83.3			1	90.9	
MEH					0.64				0.69
Slow/Very Slow	61	38.6	33	57.1			18	69.3	
Normal	71	44.9	33	60.3			16	76.3	
Rapid	26	16.5	12	65.4			6	74.2	
MPO463G>A					0.18				0.57
Variants	60	37.7	33	58.8			16	72.9	
Wild Type	99	62.3	46	59.9			24	73.2	
GSTT1					0.30				0.37
Non-null	101	64.3	52	56.9			28	70.2	
Null	56	35.7	25	66.5			12	77.9	
GSTM1					0.92				0.40
Non-null	57	36.1	26	64.1			17	69.8	
Null	101	63.9	52	57.4			23	74.9	

[1] Genotype is missing for some cases due to insufficient blood volume or assay failure

[2] Kaplan-Meier survival estimate

[3] Log-rank test

Table 4

Results of Cox Proportional Hazards Regression: Predicted NAT2 Phenotype and Risk of Death from Any Cause

	No. of cases	No. of deaths	Person-years	HR[1]	95% CI[1]
NAT2 Phenotype[2]					
Slow	68	39	285.6	1.00	Reference
Fast	78	34	407.3	0.64	0.40–1.04
Radiation					
Slow	21	14	76.0	1.00	Reference
Fast	16	11	70.4	0.67	0.31–1.59
Chemoradiotherapy					
Slow	21	10	102.5	1.00	Reference
Fast	32	16	159.0	1.21	0.54–2.73
No Chemotherapy/Radiotherapy					
Slow	26	15	107.1	1.00	Reference
Fast	30	7	177.9	0.26	0.10–0.66

HR, hazard ratio from Cox proportional hazards regression model, CI=confidence interval.

NAT2 phenotype is inferred from genotype as described under *Materials and Methods*.

[1] Adjusted for sex, continuous age, smoking status (ever vs. never), treatment, tumor site, and tumor stage (stage III/IV vs. stage I/II).

[2] The interaction between NAT2 and treatment is significant (P=0.04)

Table 5

Results of Cox Proportional Hazards Regression: Predicted GSTP1 Activity and Risk of Head and Neck Cancer Death

Genotype	No. of cases	No. of deaths	Person-years	HR[1]	95% CI[1]
GSTP1[2]					
Normal Activity[3]	130	37	591.4	1.00	Reference
Reduced Activity[3]	28	3	156.8	0.33	0.10–1.06
Men‡					
Normal Activity	103	33	439.0	1.00	Reference
Reduced Activity	19	1	121.5	0.12	0.02–0.91
Women					
Normal Activity	27	4	152.4	1.00	Reference
Reduced Activity	9	2	35.3	2.29	0.41–12.69

HR, hazard ratio from Cox proportional hazards regression model, CI=confidence interval.

[1] Adjusted for sex, treatment, tumor site, and tumor stage (stage III/IV vs. stage I/II).

[2] The interaction between GSTP1 and sex is significant (P=0.02).

[3] Normal activity GSTP1 genotypes are: A/A, A/B, A/D. Reduced activity genotypes are: B/B, B/C, C/C, C/D, D/D, A/C, B/D.