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GST, NAT1, CYP1A1 polymorphisms and risk of esophageal and gastric adenocarcinomas

Louise Wideroff, Ph.D., **Thomas L. Vaughan, M.D.**, **Federico M. Farin, M.D.**, **Marilie D. Gammon, Ph.D.**, **Harvey Risch, M.D.,PhD**, **Janet L. Stanford, Ph.D.**, and **Wong-Ho Chow, Ph.D.**
Division of Cancer Control and Population Sciences, National Cancer Institute, Bethesda, Maryland, USA (LW); Program in Epidemiology, Fred Hutchinson Cancer Research Center, Seattle, Washington, USA (TLV, JLS); Department of Environmental and Occupational Health Sciences, University of Washington, Seattle, Washington, USA (FMF); Department of Epidemiology, School of Public Health, University of North Carolina, Chapel Hill, North Carolina, USA (MDG); Department of Epidemiology and Public Health, Yale University School of Medicine, New Haven, Connecticut, USA (HR); Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland, USA (WHC)

Abstract

Condensed Abstract—In a population-based case-control study in the U.S., we examined risks of histologically confirmed esophageal and gastric adenocarcinomas in relation to polymorphisms of the following genes: *GSTP; GSTM1; GSTT1; NAT1;* and *CYP1A1*. For the *GSTP1 Val/Val* genotype (vs. *Ile/Ile*), the respective ORs of esophageal, cardia, and other gastric adenocarcinomas were 1.73 (0.75–4.02), 1.46 (0.57–3.73), and 1.22 (0.48–3.09), while no consistent patterns of elevated risk were associated with the null *GSTM1* or *GSTT1* genotypes, one or two copies of *NAT1*10* or **11* alleles, or *CYP1A1 Val/Val* or *Ile/Val* genotypes (vs. *Ile/Ile*).

Background—Polymorphisms in *glutathione-S-transferase* (*GST*), *N-acetyltransferase* (*NAT*) *1*, and *CYP1A1* genes have been suggested as susceptibility factors for esophageal and gastric adenocarcinomas, but have not been consistently linked to elevated risks. In a population-based case-control study, we examined risks in relation to polymorphisms of the following genes: *GSTP; GSTM1; GSTT1; NAT1;* and *CYP1A1*.

Methods—Histologically confirmed incident cases, ages 30–79, were identified in three U.S. locations. Population controls from the same catchment areas were frequency matched to expected age and sex distributions of esophageal and gastric cardia adenocarcinomas. DNA was extracted from buffy coat for PCR-based assays, with interpretable genotyping results obtained from 209 controls, 67 esophageal adenocarcinomas, 60 gastric cardia adenocarcinomas, and 56 noncardia gastric adenocarcinomas. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated among whites, adjusting for age, sex, and study center.

Results—In all histologic subgroups, ORs were somewhat elevated for the *GSTP1 Val/Val* genotype (vs. *Ile/Ile*), although 95% CIs included 1.00. The respective ORs for esophageal, cardia, and other gastric adenocarcinomas were 1.73 (0.75–4.02), 1.46 (0.57–3.73), and 1.22 (0.48–3.09).

Corresponding Author: Louise Wideroff, Applied Research Program, Division of Cancer Control and Population Sciences, National Cancer Institute, EPN 4005, 6130 Executive Boulevard, MSC 7344, Bethesda, MD 20892-7344, USA; Telephone: 1-301-435-6823; Fax: 1-301-435-3710; E-mail Wideroff@nih.gov.

No consistent patterns of elevated risk were associated with the null *GSTM1* or *GSTT1* genotypes, one or two copies of *NAT1*10* or **11* alleles, or *CYP1A1 Val/Val* or *Ile/Val* genotypes (vs. *Ile/Ile*).

Conclusions—Additional research in larger samples is needed to further assess polymorphisms and their interactions with epidemiologic risk factors, particularly for esophageal adenocarcinoma, which has been increasing markedly in incidence.

Keywords

polymorphism; genetic; cancer risk; gastrointestinal cancer; adenocarcinoma; esophageal; adenocarcinoma; gastric; epidemiology

Introduction

Adenocarcinomas of the esophagus and stomach have diverse incidence patterns and risk factors [1], but cigarette smoking has been associated with both in some studies [2–4]. Polymorphisms in the *glutathione-S-transferase* (*GST*) and *N-acetyltransferase* (*NAT*) *1* genes, which are involved in Phase II detoxification of certain tobacco carcinogens, have been suggested as susceptibility factors but have not been consistently linked to elevated risks [5]. Similarly, *CYP1A1*, a cytochrome P450 gene encoding Phase I detoxification enzymes also involved in tobacco metabolism, has been inconsistently associated with elevated cancer risks across different ethnic populations [6].

To further understand these associations, in a population-based case-control study of upper-digestive tract cancers, we examined the risk of esophageal, gastric cardia, and other gastric adenocarcinomas in relation to polymorphisms in the following genes: *GSTP1* (chromosome 11q13); *GSTM1* (1p13.3); *GSTT1* (22q11.2); *NAT1* (8p22); and *CYP1A1* (15q22).

Materials and Methods

In 1993–95, histologically confirmed cases ages 30–79 years were identified by rapid ascertainment in three U.S. locations following Institutional Review Board approvals at the collaborating institutions [2], and written informed consent from each subject. Controls were selected from the same catchment areas by random-digit dial if under age 65 years or from the Medicare sampling frame if 65 or older. Cases and controls were frequency matched to expected age and sex distributions of esophageal and gastric cardia adenocarcinomas. The New Jersey study center also matched on race (white or non-white). Subjects in two of three geographic locations (western Washington and 9 of the 15 participating counties in New Jersey) were asked to provide 30 ml blood samples at the time of interview. About 30% of cases and 4% of controls died prior to interview, and samples were obtained from approximately two-thirds of both interviewed cases and controls. The questionnaire ascertained demographic characteristics and information about exposures, including lifestyle factors, height and weight, medication, and medical history.

DNA was extracted from buffy coat for genotyping. The presence of the *GSTM1* and *GSTT1* alleles, as well as the *GSTM1*0/*0*, and *GSTT1 *0/*0* genotypes, was detected by polymerase chain reaction [7,8], and a TaqMan assay was used to differentiate the *GSTP1 Val/Val*, *GSTP1 Ile/Val*, and *GSTP1 Ile/Ile* genotypes [8,9]. A PCR/DNA sequencing-based assay, using PCR and dye terminator-cycle sequencing, was used to detect the genetic polyadenylation variants within the 3′ flanking region of the *NAT1* gene. Amplification using specific oligonucleotide primers [10] and optimized thermal cycling conditions yielded a single 345 base-pair PCR product. Alignment of the DNA sequence with the published wild type sequence [11] using DNA sequence alignment software-enabled identification of the *NAT1*3*, *NAT1*10*, and *NAT1*11* alleles. Absence of these three

variants is hereinafter referred to as *NAT1*4+*, which includes the wild type **4* plus new variants not detected by the assay.

The *CYP1A1 Ile462Val* alleles were identified using an oligonucleotide ligation-based genotyping assay [12]. The PCR primers utilized in this assay were as follows:

Forward	primer: 5′-gTCTCCCTCTggTTACAggA-3′
Reverse	primer: 5′-gAAAgACCTCCCAgCgggCA-3′

The reporter oligonucleotide probes were as follows:

Common reporter probe:	5′-TTgCCCgCTgggAggTCTTTC-3′
Wildtype allele probe:	5′-ggAAgTgTATCggTgAgACCA-3′
Mutant allele probe:	5′-ggAAgTgTATCggTgAgACCg-3′

The *Ile/Val* and *Val/Val* categories were collapsed for analysis due to insufficient numbers of the *Val/Val* genotype.

Among whites who provided blood, interpretable genotyping results were obtained for up to 209 controls (98%), 67 cases (100%) with esophageal adenocarcinoma, 60 (98%) with gastric cardia adenocarcinoma, and 56 (98%) with noncardia gastric adenocarcinoma, although final numbers differed slightly by assay (see Table 1). Numbers of nonwhites (13, 2, 1, and 12, respectively) were too small to obtain separate risk estimates, and the distribution of genotypes may vary by race [13]. Thus, these individuals were excluded from analysis.

For genotypes with three or more categories (i.e., *NAT1* and *GSTP1*), tests for Hardy-Weinberg equilibrium in controls were conducted using SAS/GENETICS Proc Allele (SAS 9 product documentation available from http://v9doc.sas.com/sasdoc/). In unconditional logistic regression analysis, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated, adjusting for age as a continuous variable, sex, and study center. Alcohol consumption, smoking, and body mass index (BMI) did not alter the associations with polymorphisms, and therefore, were not adjusted for in the models. Separate models containing interaction terms were used to test for interactions of genotype with smoking (ever vs. never), age (<60 vs. ≥60 years), and sex. An esophageal adenocarcinoma model also included an interaction term for genotype and BMI (<median vs. ≥ median, sex-specific). Analyses with pack-year, age, and BMI quartiles did not alter the conclusions, therefore we collapsed the variables.

Results

The respective median ages for controls and esophageal, gastric cardia, and other cardia adenocarcinoma cases were 66, 63, 66, and 67 years. Among controls, women were less likely than men to have blood available for genotyping (35% vs. 50%; p=.01). Otherwise, DNA availability did not differ by risk factor distribution between histologic subgroups of cases and controls.

There was no departure from Hardy-Weinberg equilibrium for *GSTP1* (p=0.76) or *NAT1* (p=0.87) in control subjects. As shown in Table I, there were no consistent patterns of elevated risk associated with the null *GSTM1* or *GSTT1* genotypes nor with one or two copies of *NAT1*10* or **11* alleles (whereas the *NAT1*3* allele was not detected in any cases or controls). In all histologic subgroups, ORs were somewhat elevated for the *GSTP1 Val/Val* genotype (vs. *Ile/Ile*), although 95% CIs included 1.00. The respective ORs for

esophageal, cardia, and other gastric adenocarcinomas were 1.73 (95% CI=0.75–4.02), 1.46 (0.57–3.73), and 1.22 (0.48–3.09). Associations were also null for *CYP1A1 Val/Val* and *Ile/Val* genotypes. There were no statistically significant interactions of genotype with the major risk factors examined (data not shown).

Discussion

This study found no evidence that the risk of adenocarcinomas of the esophagus or gastric cardia or noncardia stomach varies with *GSTP1*, *GSTM1*, or *GSTT1* genotype, nor does it vary with the *NAT1* or *CYP1A1* polymorphisms examined. These results are consistent with most upper-digestive cancer studies of *GSTT1* and some studies of *GSTP1*, *GSTM1*, *NAT1*, and *CYP1A1*. Previous gastric cancer studies generally found no association with *GSTP1* variants, whereas about one-half reported associations with *GSTM1* [5], and one reported an inverse age-dependent association with *GSTT1* [14]. Associations of esophageal cancer with *GSTM1*, *GSTP1*, and *GSTT1* were also equivocal [15–21]. The *NAT1*10* variant has been associated with elevated and decreased risks of gastric adenocarcinoma [22], whereas its etiologic role in esophageal cancer remains unexplored. The *CYP1A1* variant examined here has been associated with esophageal cancer in Chinese, but not Japanese populations [6,23]. Possible explanations for contrasting study results include sample size and power differences, ethnic variation, and random scatter around the null hypothesis.

The ORs in this study may reflect true null associations or low statistical power due to limited availability of blood for genotyping. For example, with the available sample size, this study has a power of .70 (allowing for a two-sided .05 Type I error rate) to detect a true OR of 2.66 for esophageal adenocarcinoma among individuals with the GSTP1 Val/Val genotype (vs. Ile/Ile). Detectable ORs of cardia and noncardia gastric adenocarcinoma are 2.94 and 2.82, respectively. Given the exploratory purpose of this study, however, the results contribute to the limited literature on genetic polymorphisms for these cancers and suggest that further consideration of the GSTP1 association may be warranted. Given the small numbers, associations with the *CYP1A1 Val/Val* genotype, which is rare in most populations [6], could not be assessed. An additional limitation may be potential selection bias related to DNA availability. Specifically, blood was not collected from study subjects who died before interview, which is often a limiting factor in case-control studies of cancers with short survival time. Also, one-third of cases and controls approached for blood collection did not provide samples, and female controls were significantly less likely to provide blood than male controls. Strengths of this study include the population-based case-control design and assessment of three histologic subtypes. Also to our knowledge, it is the first study to evaluate *NAT1* polymorphisms in esophageal adenocarcinoma. In this regard, since the *NAT1* genotyping associated with this study was completed, many other *NAT1* polymorphisms are now listed in various SNP databases (Entrez SNP available from http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=snp).

Further research in larger samples is needed to assess interactions of additional genetic polymorphisms and epidemiologic risk factors, particularly for esophageal adenocarcinoma, which has been increasing markedly in incidence [24,25].

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Table 1

Adjusted[1] Odds Ratios and 95% Confidence Intervals of Esophageal and Gastric Adenocarcinoma Among Whites, by *Glutathione-S-Transferase*, *NAT1*, and *CYP1A1* Genotypes

		Esophageal Adenocarcinoma			Cardia Adenocarcinoma			Other Gastric Adenocarcinoma		
Genotype	**Controls n**	**Cases n**	**OR**	**95% CI**	**Cases n**	**OR**	**95% CI**	**Cases n**	**OR**	**95% CI**
GSTT1										
Allele(s) present	173	59	1.00	---	46	1.00	---	49	1.00	---
Null	35	8	0.77	0.33–1.78	14	1.52	0.75–3.08	7	0.72	0.30–1.75
GSTM1										
Allele(s) present	87	30	1.00	---	29	1.00	---	26	1.00	---
Null	121	37	0.98	0.55–1.74	31	0.77	0.43–1.38	30	0.79	0.43–1.43
GSTP1										
Ile/Ile	91	32	1.00	---	24	1.00	---	28	1.00	---
Ile/Val	94	23	0.71	0.38–1.32	27	1.09	0.58–2.03	19	0.63	0.33–1.22
Val/Val	21	12	1.73	0.75–4.02	8	1.46	0.57–3.73	8	1.22	0.48–3.09
NAT1										
*4/*4+2	120	37	1.00	---	37	1.00	---	39	1.00	---
*10/*any (except	72	26	1.13	0.62–2.04	19	0.85	0.45–1.59	13	0.53	0.26–1.07
*10/*10	5	2	1.20	0.22–6.75	1	0.65	0.07–5.78	2	1.14	0.21–6.21
All others4	12	2	0.47	0.10–2.29	3	0.80	0.21–3.00	2	0.51	0.11–2.40
CYP1A1m2										
Ile/Ile	195	64	1.00	---	52	1.00	---	54	1.00	---
Ile/Val or Val/Val	14	3	0.69	0.19–2.55	8	2.24	0.88–5.67	2	0.49	0.11–2.26

[1] Adjusted for age as a continuous variable, study center, and sex.

[2] Includes cases and controls without any *3, *10, or *11 alleles (i.e., *4+ represents alleles not identified by the NAT1 assay used in the study).

[3] Includes controls with *10/*11 (n=2) and *4+/*10 (n=70); esophageal adenocarcinoma cases with *10/*11 (n=1) and *4+/*10 (n=25); cardia adenocarcinoma cases with *4+/*10 (n=19); other gastric adenocarcinoma cases with *10/*11 (n=1) and *4+/*10 (n=12).

[4] Includes controls with *4+/*11 (n=11) and *11/*11 (n=1); esophageal adenocarcinoma cases with *4+/*11 (n=1) and *11/*11 (n=1); cardia adenocarcinoma cases with *4+/*11 (n=3); other gastric adenocarcinoma cases with *4+/*11 (n=2).