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Cigarette smoking, body mass index, gastro-esophageal reflux disease, and non-steroidal anti-inflammatory drug use and risk of subtypes of esophageal and gastric cancers by P53 overexpression

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Abstract

A number of risk factors for esophageal and gastric cancers have emerged, yet little is known whether risk factors map to molecular tumor markers such as overexpression of the tumor suppressor *TP53*. Using a US multicenter, population-based case-control study (170 cases of esophageal adenocarcinomas, 147 gastric cardia adenocarcinomas, 220 non-cardia gastric adenocarcinomas, and 112 esophageal squamous cell carcinomas), we examined whether the risk associated with cigarette smoking, body mass index (BMI), gastroesophageal reflux disease (GERD), and non-steroidal anti-inflammatory drug (NSAID) use varied by P53 overexpression. We defined P53 overexpression through immunohistochemistry of paraffin-embedded tumor tissues, using cutpoints based on percent of cells positive. Polytomous logistic regression was used to assess differences between each case group (defined by tumor subtype and P53 expression) and the control group by risk factors. The proportion of cases overexpressing P53 by tumor subtype was 72% for esophageal adenocarcinoma, 69% for gastric cardia adenocarcinoma, 52% for non-cardia gastric adenocarcinoma, and 67% for esophageal squamous cell carcinoma. For most tumor subtypes, we found little difference in risk factors by tumor P53 overexpression. For non-cardia gastric cancer however, an association with cigarette smoking was suggested for tumors that do not overexpress P53, while larger BMI was related with tumors that overexpress P53 versus no overexpression. Overall, this study did not find a clear relationship between P53 protein overexpression and known risk factors for subtypes of esophageal and gastric cancers. Further research with these tumors is needed to identify molecular markers associated with variations in the risk factor profiles.

INTRODUCTION

The P53 protein is a critical regulator of processes important in carcinogenesis, including cell cycle control, DNA repair, and apoptosis (1). P53 is tightly regulated, normally expressed at low levels; however, mutation of the gene encoding P53 (*TP53*) can result in abnormal P53 protein accumulation which has been associated with neoplasia (2). There is evidence that the nature and distribution of *TP53* mutations may reflect exposure to etiologic factors (3,4). For example, chemical carcinogens in tobacco smoke generally produce transversion mutations, while endogenous mutagens, such as nitric oxide, cause mostly transition mutations (5). Furthermore, a strong correlation exists between missense mutations in *TP53* and high levels of protein accumulation (5), so P53 immunohistochemistry has been used as a marker for mutation. Accordingly, we examined subtypes of esophageal and gastric cancers, to determine whether P53 overexpression may also vary by risk factors. We have previously shown differences in non-steroidal anti-inflammatory drug (NSAID) use among individuals with esophageal and gastric cardia adenocarcinomas that overexpress cyclin D1 when compared to tumors that do not overexpress this marker (6). P53 overexpression has been described in subtypes of esophageal and gastric cancers (7–12), but its relation to risk factors is not well-defined.

A number of risk factors have been identified for esophageal and gastric cancer, with variations by subtype (i.e. esophageal adenocarcinoma, esophageal squamous carcinoma, gastric cardia adenocarcinoma, non-cardia adenocarcinomas). Cigarette smoking has been associated with increased risk, while use of NSAIDs appears to be protective for all four subtypes (13–17). In contrast, gastroesophageal reflux (GERD), defined in terms of the frequency and severity of heartburn symptoms, and elevated body mass index (BMI) have been associated with elevated risk of esophageal adenocarcinoma, while gastric cardia adenocarcinoma has been associated to a lesser extent only with elevated BMI (18–21). Here we examine the association of these risk factors with subtypes of esophageal and gastric cancers, stratified by P53 expression status, in a multicenter study of 649 cases and 695 controls across the United States, to test the hypothesis that P53 overexpression is the target mechanism by which the known risk factors smoking, NSAID, GERD, and BMI act in subtypes of esophageal and gastric cancers.

METHODS

Study population

The methods for this population-based case-control study have been reported in detail elsewhere (14). Briefly, residents newly diagnosed with invasive esophageal or gastric cancers at ages 30–79 years in Connecticut (from February 1, 1993, to January 31, 1995), New Jersey (from April 1, 1993, to November 30, 1994), and western Washington state (from March 1, 1993, to February 28, 1995) were identified through rapid reporting systems. Population-based control subjects were selected by random digit dialing (22) for those under 65 years of age and from the Health Care Financing Administration files for those 65 years of age or older. Cases and controls were frequency matched to expected age and sex distributions of esophageal and gastric cardia adenocarcinomas. In New Jersey, cases and controls also were matched on race. Classification of cases by site of origin and histology was determined by two pathologists through standardized review of pathology materials and reports from surgery, endoscopy, and radiology.

Risk factor information

Methods for data collection have been previously described (14,17,20). Briefly, in-person interviews were conducted with control subjects and cases, as well as next of kin of deceased cases, for information on demographic characteristics, cigarette smoking and alcohol use, other

beverage consumption, medical history, use of medications, diet, and occupational history. Proxy interviews were conducted for 30% of the cases and 3% of controls. For cigarette smoking, never smoking was defined as having smoked less than 100 cigarettes lifetime or as having smoked less than one cigarette per day for any 6-month period. A former smoker was defined as having stopped smoking 2 or more years before the interview (14). NSAID (or aspirin) users were defined as persons having taken NSAIDs (or aspirin) at least once per week for 6 months or more (17). To determine the number of episodes of severe heartburn as a symptom for GERD, subjects were asked “For how many months or years in total did (you/s/he) have severe heartburn”. The respondents indicated the number of months or years or that they did not know. In the tables presented, GERD was defined as more than 1 reported GERD episode per year (20). In addition, we evaluated a more stringent definition of GERD as 13 episodes or more per year, but this did not change interpretation of the findings.

Tumor immunohistochemistry

Archived tumor blocks with adequate tissue for immunohistochemical analyses were acquired for 649 (56.8%) of the 1130 cancer cases. As reported previously (6), the availability of tumor tissue varied little by tumor subsite, histology, or stage at diagnosis (data not shown). In addition, the availability of tumor tissue did not vary by direct versus proxy interview. The methods for P53 protein analysis have also been described previously (23). In brief, the P53 DO-7 monoclonal antibody (1:50; DAKO Corp., Santa Barbara, CA) was used on dewaxed formalin-fixed, paraffin sections of tumor, utilizing microwave antigen retrieval (24). The immunohistochemical protocol was performed by a Ventana Automated Immunostainer (Ventana Corp., Tucson, AZ) using a 32-minute incubation of the primary antibody and avidin-biotin complex immunoperoxidase technique (25). Stains for the slides were performed in multiple batches in the same lab and a positive and negative control was included to be sure results were consistent across batches. Nuclear P53 protein staining was assessed by a study pathologist. The percentage of stained tumor cells was coded as a 5-level ordered categorical variable where: 0= negative 1= <10%; 2=10–50%; 3=51–90%, and 4=>90% cells stained positive.

Statistical methods

Odds ratios (OR) and 95% confidence intervals (CI) were estimated for P53+ cases and P53– cases separately by comparison to controls in unordered polytomous logistic regression models (26). For the purposes of this study, P53 overexpression was defined as tumors having nuclear staining in 10% of cells or more, and moderate or strong intensity score of staining. We also performed sensitivity analyses on the final models using more stringent levels of percent positive cells. Such models with alternative cutpoints did not change the interpretation of our findings. For ease of presentation, we refer to these cases with protein overexpression as P53 + cases throughout the manuscript; those cases with less than 10% positive nuclear staining are referred to as P53– cases. Five models were evaluated for the following risk factors: cigarette smoking (never, former, current), BMI (< 25, 25–30, 30+), GERD (No/Yes), any NSAID use (non-users of any NSAID, any type), and aspirin use (non-users of any NSAID, aspirin use only). ORs and 95% CIs were computed for P53+ cases relative to P53– cases to evaluate the significance of the differences in risk by P53 overexpression. The inclusion of the other risk factors for adjustment (cigarette smoking, BMI, GERD, NSAID use, alcohol drinking, and education) was also performed in the models. Any variable that changed the model parameter estimates for P53 overexpression by 10% or more was included in the final models for each risk factor (26). To account for non-linear relationships with age, age and age squared (age²) terms were included in the regression models. In addition, models including and excluding proxy interview status were considered, but they did not appreciably change any of the results and are not reported here. Lastly, since P53 overexpression has been suggested

in other tumors to be related to stage, we performed stratified analysis by tumor stage at diagnosis (local/regional versus distant/advanced stage).

RESULTS

Among cases with tumor slides for immunohistochemistry staining, 64% were found to overexpress P53, with slight variations by tumor subtype: 72% for esophageal adenocarcinoma, 69% for gastric cardia adenocarcinoma, 52% for non-cardia gastric adenocarcinoma, and 67% for esophageal squamous cell carcinoma (Table 1). Within tumor subtypes, P53 overexpression did not vary consistently by study center, age, race, or sex. P53 overexpression (P53+) was more frequent with advanced stage at diagnosis for esophageal and gastric cardia adenocarcinomas, although the differences did not reach statistical significance (Table 1).

The associations with cigarette smoking for P53+ and P53- tumors were similar for esophageal and gastric cardia adenocarcinomas (Table 2). Cigarette smoking was also associated with elevated risks of both non-cardia gastric adenocarcinoma and esophageal squamous cell carcinoma regardless of P53 status. However, the risks were generally greater among P53- tumors (e.g., in non-cardia gastric cancer, OR for P53+ versus P53- case/case differences = 0.49, 95% CI = 0.25–0.96 and OR = 0.62, 95% CI = 0.29–1.35 for former smokers and current smokers, respectively, relative to never smokers). A high excess risk for all four subtypes was observed among former and current smokers, but current smokers tended to have higher risks for all four cancer types.

As expected, excess BMI was significantly associated with increased risks of esophageal adenocarcinoma and to a lesser extent, gastric cardia adenocarcinoma (Table 3). The elevated risks did not vary significantly by P53 overexpression, although adenocarcinomas regardless of subsite, generally had higher risks among P53+ cases than P53- cases when compared with controls. For example, for non-cardia gastric cancer, the OR comparing P53+ to P53- cases was 1.16 (95% CI = 0.64–2.10) for those with BMI 25–30 and 2.5 (95% CI = 0.98–6.40) for those with BMI >30. Risks for esophageal squamous cell carcinoma tended to decrease with increasing BMI, and with no significant differences between P53+ and P53- cases.

A self-reported history of GERD was associated with an elevated risk only for esophageal adenocarcinomas, with the risk estimates similar for P53+ and P53- tumors (Table 4). The risk for gastric cardia adenocarcinoma appeared to be inversely associated with GERD, but only among P53+ cases. GERD was not associated with risk for non-cardia gastric adenocarcinoma, but was inversely related to esophageal squamous cell carcinoma, regardless of P53 overexpression. Furthermore, a more stringent definition of GERD (13 episodes/year) did not alter the conclusions (data not shown).

In our study, use of NSAIDs was associated with a reduced risk for all subtypes of esophageal and gastric cancers as previously reported (6). The inverse associations were comparable for P53+ and P53- tumors except for gastric cardia adenocarcinomas which showed an inverse association for P53+ cases and a positive association for P53- cases (Table 5). However, none of the associations reached statistical significance.

Supplemental analyses using more stringent cutpoints for P53 positivity (50% positive cells) did not materially alter the findings. Stratification by tumor stage at diagnosis (localized/regional and distant/metastatic) also did not reveal any consistent differences in risk patterns by P53 overexpression.

DISCUSSION

The present study examined the risk factors associated with subtypes of esophageal and gastric cancers that were characterized by P53 overexpression status. The preponderance of P53 overexpression in all subtypes of esophageal and gastric cancers in our study is consistent with previous reports (7–12). However, we did not observe consistent differences in risk patterns by P53 overexpression status for tumor subtypes.

Cigarette smoking is a well-documented risk factor for esophageal and gastric cancers, although the magnitude of association varies for tumor subtypes (14,27). In our study, smoking-related risk for esophageal and gastric cardia adenocarcinomas did not vary by P53 overexpression status, but the risk for non-cardia gastric adenocarcinoma and esophageal squamous cell carcinoma tended to be higher among P53⁻ than P53⁺ tumors. Esophageal squamous cell carcinomas among smokers have been reported with excess transversion *TP53* mutations, which is consistent with the mutation signature of tobacco carcinogens, such as polycyclic aromatic hydrocarbons and nitrosamines (28,29). Our failure to observe a greater smoking-related risk for P53⁺ cases may be due to unstable risk estimates since only 11% (12/112) of esophageal squamous cell carcinomas reported never smoking and only one P53⁻ tumor was a never smoker. In contrast, the *TP53* mutation signatures in esophageal and gastric adenocarcinomas feature transition mutations at CpG sites, which is consistent with the role of endogenous mutagens, such as oxyradicals and nitrogen oxyradicals, rather than exogenous carcinogens as found in cigarette smoke (4). This is consistent with the similar smoking-related risks we observed for P53⁺ and P53⁻ cases of esophageal and gastric adenocarcinomas, and with the hypothesis that risk factors other than smoking induce P53 inactivation (4,29).

Elevated BMI has been associated with excess risk of esophageal adenocarcinoma, and to a lesser extent with gastric cardia adenocarcinoma (18,19,21). In our analysis, BMI-related risks did not vary consistently or significantly by P53 overexpression, although the increases in risk of adenocarcinomas tended to be more pronounced for P53⁺ tumors than P53⁻ tumors. While the mechanism linking obesity to esophageal adenocarcinomas is unclear, a recent study of Barrett's esophagus, a precursor state, revealed loss of the chromosomal region (17p) where *TP53* resides among individuals with increased waist-to-hip ratio, another proxy for obesity besides BMI (30). In addition, mutation of *TP53* has been related to dietary factors, including high intake of fat, protein, or nitrites in gastric cancers (31), suggesting that differences in BMI-related risk by P53 expression status may be in part a function of nutrient intake.

GERD has been associated with increased risk for esophageal adenocarcinomas (21), and may act through a chronic inflammatory process that increases the likelihood of *TP53* mutation in esophageal tissue (32). However, the association with GERD in our study did not vary significantly by P53 overexpression, perhaps because not all *TP53* mutations result in P53 overexpression (8), or because P53 is not central to the multi-stage progression from GERD to adenocarcinoma.

The inverse associations with NSAID use for the tumor subtypes in our study did not vary consistently by P53 expression. In a recent cross-sectional study of 429 persons with Barrett's esophagus, the odds ratio for current regular NSAID users compared with neverusers was 0.3 (95% CI, 0.1–0.7) among individuals with loss of heterozygosity in chromosome 17p (30), suggesting that NSAID may suppress inactivation of the *TP53* locus. Given the lack of an association between NSAID use and P53 overexpression of the tumor subtypes in our study, other molecular mechanisms such as cyclin D overexpression(6) may be the pathway by which this protective factor acts.

This study took advantage of previous work demonstrating that mutations of *TP53* often result in overexpression of P53 (2,33). However, there are limitations to using protein expression as a marker for *TP53* mutation, since it has been shown that 30–35% of tumors harboring *TP53* mutations do not result in protein accumulation (34). Since the prevalence of P53 overexpression increases with advancing tumor stage at diagnosis, survival bias may occur if any of the risk factors are related also to survival (35). Our results were adjusted for proxy interviews, conducted primarily for deceased patients or those too ill to be interviewed. Furthermore, stratification by tumor stage at diagnosis yielded comparable results, arguing against a systematic bias due to survival.

In summary, this population-based study examined the association of risk factors for subtypes of esophageal and gastric cancers by P53 overexpression. There was no clear evidence of case heterogeneity by P53 overexpression except for suggestive differences in the risk association of non-cardia gastric adenocarcinoma in relation to cigarette smoking and BMI. Given the differences in risk factors by morphologic subtypes of esophageal and gastric cancer, further studies with sufficient power to also include molecular subtypes should provide some insights into causal pathways as targets for prevention.

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Table 1

Characteristics by tumor type and P53 overexpression

	Esophageal adenocarcinoma N=170	P value	Gastric Cardia adenocarcinoma N=147	P value	Non-cardia gastric adenocarcinoma N=220	P value	Esophageal squamous cell carcinoma N=112	P value
P53 overexpression	122 (71.8%)		102 (69.4%)		114 (51.8%)		75 (67.0%)	
Study center								
Connecticut	23 (67.7%)		20 (57.1%)		27 (48.2%)		23 (63.9%)	
New Jersey	72 (74.2%)		53 (70.7%)		66 (55.0%)		41 (70.7%)	
Washington	27 (69.2%)	0.71	29 (78.4%)	0.14	21 (47.7%)	0.58	11 (61.1%)	0.67
Sex								
Male	98(71.5%)		91(69.5%)		85(53.1%)		56(62.9%)	
Female	24(72.7%)	0.89	11(68.75%)	0.95	29(48.3%)	0.53	19(82.6%)	0.07
Age								
mean (std)	64.1(11.2)	0.31	62.7(11.0)	0.40	66.0(9.8)	0.31	65.8(9.7)	0.55
Race								
White	120(71.4%)		99(70.2%)		95(51.1%)		60(69.8%)	
Non-whites	2 (100%)	1.00	3(50.0%)	0.37	19(55.9%)	0.71	15(57.7%)	0.25
Tumor Stage								
in situ or localized	26(65.0%)		7(58.3%)		14(43.75%)		16(80.0%)	
regional	33(76.7%)		46(67.65%)		60(55.1%)		28(66.7%)	
distant or metastatic	43(79.6%)	0.25**	37(74.0%)	0.53**	30(46.15%)	0.37**	15(88.2%)	0.18**
unknown	20(60.6%)		12(70.6%)		10(71.4%)		16(48.5%)	

* from Chi-Square test

** Chi-square test excluding those specimens with unknown stage.

Adjusted^a odds ratios (OR) and 95% confidence intervals (CI) for P53 overexpression (P53+) and P53 negative (P53-) esophageal and gastric cancer, in relation to cigarette smoking by tumor type among men and women in Connecticut, New Jersey, and Washington 1993-1995

Table 2

Smoking Status	controls (N=695)	P53+ cases			P53- cases			Ratio of P53+/P53-		
		(N=413)	OR	95%CI	(N=236)	OR	95% CI	OR	95% CI	p value
Esophageal adenocarcinoma										
Never	244	26	1.00	---	10	1.00	---	---	---	---
Ever	451	96	2.23	1.33-3.73	38	2.03	0.95-4.32	1.10	0.47-2.58	0.83
Former	296	61	2.12	1.23-3.67	21	1.64	0.73-3.72	1.29	0.52-3.23	0.58
Current	155	35	2.43	1.32-4.46	17	2.83	1.20-6.70	0.86	0.33-2.26	0.76
Gastric cardia adenocarcinoma										
Never	244	20	1.00	---	8	1.00	---	---	---	---
Ever	451	82	2.39	1.37-4.17	37	2.52	1.10-5.76	0.95	0.37-2.44	0.91
Former	296	51	2.20	1.22-3.97	19	1.84	0.75-4.49	1.19	0.43-3.28	0.73
Current	155	31	2.73	1.44-5.18	18	3.99	1.60-9.93	0.69	0.24-1.95	0.48
Non-cardia gastric adenocarcinoma										
Never	244	36	1.00	---	22	1.00	---	---	---	---
Ever	451	78	1.43	0.89-2.30	84	2.68	1.55-4.63	0.53	0.28-1.01	0.05
Former	296	48	1.28	0.77-2.16	58	2.63	1.48-4.68	0.49	0.25-0.96	0.04
Current	155	30	1.72	0.96-3.06	26	2.75	1.42-5.30	0.62	0.29-1.35	0.23
Esophageal squamous cell carcinoma										
Never	244	11	1.00	---	1	1.00	---	---	---	---
Ever	451	64	3.28	1.61-6.67	36	17.29	2.29-130.63	0.19	0.02-1.56	0.12
Former	296	31	2.21	1.03-4.75	10	6.84	0.84-55.47	0.32	0.04-2.87	0.31
Current	155	33	5.69	2.60-12.42	26	42.19	5.43-327.58	0.14	0.02-1.14	0.07
Former	296	31	1.00	---	10	1.00	---	---	---	---
Current	155	33	2.64	1.43-4.84	26	6.26	2.69-14.58	0.42	0.17-1.06	0.07

^a Adjusted for center, age, race, proxy interview, and gender. Other variables GERD, NSAID use, education, BMI, and alcohol, use were not found to change parameter estimates significantly.

Adjusted^a odds ratios (OR) and 95% confidence intervals (CI) for P53 overexpression (P53+) and P53 negative (P53-) esophageal and gastric cancer, in relation to Body Mass Index (BMI) by tumor type among men and women in Connecticut, New Jersey, and Washington 1993-1995

Table 3

BMI	controls (N=695)	P53+ cases			P53- cases			Ratio of P53+/P53-		
		(N=412)	OR	95%CI	(N=236)	OR	95% CI	OR	95% CI	p value
Esophageal adenocarcinoma										
<25	341	40	1.00	---	19	1.00	---	---	---	---
25-30	289	57	2.16	1.30-3.56	21	1.65	0.83-3.29	1.31	0.60-2.84	0.50
30+	62	25	5.32	2.75-10.29	8	3.34	1.30-8.62	1.59	0.58-4.39	0.37
Gastric cardia adenocarcinoma										
<25	341	39	1.00	---	14	1.00	---	---	---	---
25-30	289	45	1.40	0.86-2.27	25	2.10	1.03-4.31	0.67	0.30-1.50	0.33
30+	62	18	2.44	1.23-4.86	6	2.16	0.76-6.18	1.13	0.36-3.56	0.84
Non-cardia gastric adenocarcinoma										
<25	341	51	1.00	---	56	1.00	---	---	---	---
25-30	289	45	1.51	0.94-2.45	42	1.31	0.81-2.11	1.16	0.64-2.10	0.63
30+	62	18	2.46	1.25-4.85	8	0.99	0.42-2.29	2.50	0.98-6.40	0.06
Esophageal squamous cell carcinoma										
<25	341	42	1.00	---	19	1.00	---	---	---	---
25-30	289	26	0.94	0.53-1.67	16	1.04	0.47-2.29	0.91	0.37-2.20	0.83
30+	62	6	0.85	0.32-2.25	2	0.48	0.10-2.35	1.79	0.31-10.27	0.52

^a Adjusted for center, age, race, gender, gerd, NSAID use, proxy interview, and education. Other variables alcohol, and cigarette smoking were not found to change parameter estimates significantly.

Adjusted^a odds ratios (OR) and 95% confidence intervals (CI) for P53 overexpression (P53+) and P53 negative (P53-) esophageal and gastric cancer, in relation to Gastrointestinal reflux disease (GERD) by tumor type among men and women in Connecticut, New Jersey, and Washington 1993-1995

Table 4

GERD	controls (N=695)	P53+ cases			P53- cases			Ratio of P53+/P53-		
		(N=413)	OR	95%CI	(N=236)	OR	95% CI	OR	95% CI	p value
Esophageal adenocarcinoma										
No	373	47	1.00	---	20	1.00	---	---	---	---
Yes	322	75	2.68	1.70-4.22	28	2.26	1.19-4.29	1.19	0.58-2.42	0.64
Gastric cardia adenocarcinoma										
No	373	69	1.00	---	25	1.00	---	---	---	---
Yes	322	33	0.56	0.35-0.90	20	0.99	0.52-1.89	0.57	0.27-1.21	0.14
Non-cardia gastric adenocarcinoma										
No	373	68	1.00	---	58	1.00	---	---	---	---
Yes	322	46	1.01	0.65-1.57	48	1.19	0.76-1.86	0.84	0.49-1.47	0.57
Esophageal squamous cell carcinoma										
No	373	59	1.00	---	30	1.00	---	---	---	---
Yes	322	16	0.47	0.25-0.86	7	0.40	0.17-0.98	1.15	0.41-3.24	0.79

^a Adjusted for center, age, race, gender, proxy interview, and NSAID. Other variables alcohol, cigarette smoking, BMI, and education were not found to change parameter estimates significantly.

Adjusted^a odds ratios (OR) and 95% confidence intervals (CI) for P53 overexpression (P53+) and P53 negative (P53-) esophageal and gastric cancer, in relation to use of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) by tumor type among men and women in Connecticut, New Jersey, and Washington 1993-1995

Table 5

NSAID use	controls (N=695)	P53+ cases			P53- cases			Ratio of P53+/P53-		
		(N=404)	OR	95%CI	(N=231)	OR	95% CI	OR	95% CI	p value
Esophageal adenocarcinoma										
None	410	77	1.00	---	30	1.00	---	---	---	---
Any type	282	41	0.60	0.38-0.97	17	0.59	0.30-1.15	1.03	0.49-2.15	0.94
None	410	77	1.00	---	30	1.00	---	---	---	---
Aspirin	222	35	0.70	0.42-1.17	14	0.61	0.29-1.25	1.16	0.52-2.56	0.72
Gastric cardia adenocarcinoma										
None	410	65	1.00	---	21	1.00	---	---	---	---
Any type	282	35	0.80	0.49-1.28	23	1.50	0.77-2.92	0.53	0.25-1.13	0.10
None	410	65	1.00	---	21	1.00	---	---	---	---
Aspirin	222	29	0.86	0.51-1.44	17	1.42	0.68-2.95	0.61	0.26-1.39	0.24
Non-cardia gastric adenocarcinoma										
None	410	88	1.00	---	73	1.00	---	---	---	---
Any type	282	26	0.36	0.22-0.60	32	0.51	0.31-0.84	0.71	0.38-1.33	0.28
None	410	88	1.00	---	73	1.00	---	---	---	---
Aspirin	222	19	0.34	0.19-0.60	27	0.54	0.31-0.93	0.62	0.31-1.25	0.18
Esophageal squamous cell carcinoma										
None	410	49	1.00	---	25	1.00	---	---	---	---
Any type	282	23	0.51	0.28-0.94	10	0.39	0.17-0.92	1.32	0.51-3.43	0.57
None	410	49	1.00	---	25	1.00	---	---	---	---
Aspirin	222	18	0.54	0.28-1.05	9	0.48	0.20-1.20	1.12	0.40-3.13	0.83

^a Adjusted for center, age, race, gender, cigarette smoking, gerd, proxy interview, and BMI. Other variables alcohol and education were not found to change parameter estimates significantly.