ORIGINAL PAPER

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

**Jonine D. Figueroa · Mary Beth Terry · Marilie D. Gammon ·
Thomas L. Vaughan · Harvey A. Risch · Fang-Fang Zhang · David E. Kleiner ·
William P. Bennett · Christine L. Howe · Robert Dubrow · Susan T. Mayne ·
Joseph F. Fraumeni Jr. · Wong-Ho Chow**

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, whereas larger BMI was related to adenocarcinomas that overexpress P53 versus no overexpression. Overall, this study did not find a clear relationship between P53 protein overexpression and the known risk factors for subtypes of

J. D. Figueroa (✉) · J. F. Fraumeni Jr. · W.-H. Chow
Division of Cancer Epidemiology and Genetics, National
Cancer Institute, Bethesda, MD, USA
e-mail: figueroaj@mail.nih.gov

J. D. Figueroa
Cancer Prevention Fellowship Program, Office of Preventive
Oncology, National Cancer Institute, National Institutes of
Health, Bethesda, MD, USA

M. B. Terry · F.-F. Zhang
Department of Epidemiology, Mailman School of Public Health,
Columbia University, New York, NY, USA

M. D. Gammon
Department of Epidemiology, School of Public Health,
University of North Carolina, Chapel Hill, NC, USA

T. L. Vaughan
Program in Epidemiology, Fred Hutchinson Cancer Research
Center, Seattle, WA, USA

T. L. Vaughan
Department of Epidemiology, School of Public Health &
Community Medicine, University of Washington, Seattle, WA,
USA

H. A. Risch · R. Dubrow · S. T. Mayne
Department of Epidemiology and Public Health, Yale University
School of Medicine, New Haven, CT, USA

D. E. Kleiner
Laboratory of Pathology, National Cancer Institute, National
Institutes of Health, Bethesda, MD 20892, USA

W. P. Bennett
Department of Thoracic Surgery, City of Hope National Medical
Center, Duarte, CA 91010, USA

C. L. Howe
Department of Pathology, Yale University School of Medicine,
New Haven, CT, USA

esophageal and gastric cancers. Further research on these tumors is needed to identify molecular markers associated with variations in the risk factor profiles.

Keywords P53 · Esophageal cancer · Body mass index · Tobacco · NSAIDs · GERD

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 and 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, whereas 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, whereas 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, whereas 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]. In brief, 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 who were 65 years of age or older. 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]. In brief, in-person interviews were conducted with control subjects and cases, as well as with the next of kin of the 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 two 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 immuno-histochemical 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 were included to ensure the 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 $0 = -$ (minus) $1 = <10\%$; $2 = 10–50\%$; $3 = 51–90\%$, 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 comparing with 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 cut-points 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 nonlinear relationships with age, age and age squared (age^2) 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 adeno-carcinoma, 69% for gastric cardia adenocarcinoma, 52% for non-cardia gastric adenocarcinoma, and 67% for esophageal squamous cell carcinoma (Table 1). Across 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 comparisons = 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.

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%)	0.99	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

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, 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

This 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 to have excess transversion TP53 mutations, which is consistent with the mutation signature of tobacco carcinogens, such as polycyclic aromatic

Table 2 Adjusted[a] odds ratios (ORs) 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

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

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. Whereas 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

Table 3 Adjusted[a] odds ratios (ORs) 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

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

Table 4 Adjusted[a] odds ratios (ORs) 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

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

expression. In a recent cross-sectional study of 429 persons with Barrett's esophagus, the OR for current regular NSAID users compared with never users 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

Table 5 Adjusted[a] odds ratios (ORs) 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

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

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]. Because 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.

References

1. Levine AJ, Momand J, Finlay CA (1991) The p53 tumour suppressor gene. Nature 351(6326):453–456. doi:10.1038/351453a0
2. Dowell SP, Wilson PO, Derias NW, Lane DP, Hall PA (1994) Clinical utility of the immunocytochemical detection of p53 protein in cytological specimens. Cancer Res 54(11):2914–2918
3. Bennett WP, Hussain SP, Vahakangas KH, Khan MA, Shields PG, Harris CC (1999) Molecular epidemiology of human cancer risk: gene-environment interactions and p53 mutation spectrum in

human lung cancer. J Pathol 187(1):8–18. doi:10.1002/(SICI) 1096-9896(199901)187:1<8::AID-PATH232>3.0.CO;2-Y

4. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P (2002) The IARC TP53 database: new online mutation analysis and recommendations to users. Hum Mutat 19(6):607–614. doi:10.1002/humu.10081

5. Soussi T, Beroud C (2001) Assessing TP53 status in human tumours to evaluate clinical outcome. Nat Rev Cancer 1(3):233–240. doi:10.1038/35106009

6. Gammon MD, Terry MB, Arber N, Chow WH, Risch HA, Vaughan TL et al (2004) Nonsteroidal anti-inflammatory drug use associated with reduced incidence of adenocarcinomas of the esophagus and gastric cardia that overexpress cyclin D1: a population-based study. Cancer Epidemiol Biomarkers Prev 13(1):34–39. doi:10.1158/1055-9965.EPI-03-0198

7. Coggi G, Bosari S, Roncalli M, Graziani D, Bossi P, Viale G et al (1997) p53 protein accumulation and p53 gene mutation in esophageal carcinoma. A molecular and immunohistochemical study with clinicopathologic correlations. Cancer 79(3):425–432. doi:10.1002/(SICI)1097-0142(19970201)79:3<425::AID-CNCR1>3.0.CO;2-H

8. Doak SH, Jenkins GJ, Parry EM, Griffiths AP, Shah V, Baxter JN et al (2003) Characterisation of p53 status at the gene, chromosomal and protein levels in oesophageal adenocarcinoma. Br J Cancer 89(9):1729–1735. doi:10.1038/sj.bjc.6601323

9. Flejou JF, Muzeau F, Potet F, Lepelletier F, Fekete F, Henin D (1994) Overexpression of the p53 tumor suppressor gene product in esophageal and gastric carcinomas. Pathol Res Pract 190(12):1141–1148

10. Gleeson CM, Sloan JM, McManus DT, Maxwell P, Arthur K, McGuigan JA et al (1998) Comparison of p53 and DNA content abnormalities in adenocarcinoma of the oesophagus and gastric cardia. Br J Cancer 77(2):277–286

11. Jovanovic I, Todorovic V, Milosavljevic T, Micev M, Pesko P, Bjelovic M et al (2005) Expression of p53 protein in Barrett's adenocarcinoma and adenocarcinoma of the gastric cardia and antrum. Vojnosanit Pregl 62(12):879–885

12. Shi ST, Yang GY, Wang LD, Xue Z, Feng B, Ding W et al (1999) Role of p53 gene mutations in human esophageal carcinogenesis: results from immunohistochemical and mutation analyses of carcinomas and nearby non-cancerous lesions. Carcinogenesis 20(4):591–597. doi:10.1093/carcin/20.4.591

13. Ahsan H, Neugut AI, Gammon MD (1997) Association of adenocarcinoma and squamous cell carcinoma of the esophagus with tobacco-related and other malignancies. Cancer Epidemiol Biomarkers Prev 6(10):779–782

14. Gammon MD, Schoenberg JB, Ahsan H, Risch HA, Vaughan TL, Chow WH et al (1997) Tobacco, alcohol, and socioeconomic status and adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 89(17):1277–1284. doi:10.1093/jnci/89.17.1277

15. Lindblad M, Rodriguez LA, Lagergren J (2005) Body mass, tobacco and alcohol and risk of esophageal, gastric cardia, and gastric non-cardia adenocarcinoma among men and women in a nested case-control study. Cancer Causes Control 16(3):285–294. doi:10.1007/s10552-004-3485-7

16. Corley DA, Kerlikowske K, Verma R, Buffler P (2003) Protective association of aspirin/NSAIDs and esophageal cancer: a systematic review and meta-analysis. Gastroenterology 124(1):47–56. doi:10.1053/gast.2003.50008

17. Farrow DC, Vaughan TL, Hansten PD, Stanford JL, Risch HA, Gammon MD et al (1998) Use of aspirin and other nonsteroidal anti-inflammatory drugs and risk of esophageal and gastric cancer. Cancer Epidemiol Biomarkers Prev 7(2):97–102

18. Chow WH, Blot WJ, Vaughan TL, Risch HA, Gammon MD, Stanford JL et al (1998) Body mass index and risk of adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 90(2):150–155. doi:10.1093/jnci/90.2.150

19. Lagergren J, Bergstrom R, Nyren O (1999) Association between body mass and adenocarcinoma of the esophagus and gastric cardia. Ann Intern Med 130(11):883–890

20. Farrow DC, Vaughan TL, Sweeney C, Gammon MD, Chow WH, Risch HA et al (2000) Gastroesophageal reflux disease, use of H2 receptor antagonists, and risk of esophageal and gastric cancer. Cancer Causes Control 11(3):231–238. doi:10.1023/A:1008913828105

21. Hampel H, Abraham NS, El-Serag HB (2005) Meta-analysis: obesity and the risk for gastroesophageal reflux disease and its complications. Ann Intern Med 143(3):199–211

22. Wakesberg J (1978) Sampling methods for random digit dialing. J Am Stat Assoc (73):40–46. doi:10.2307/2286513

23. Guinee DG Jr, Travis WD, Trivers GE, De Benedetti VM, Cawley H, Welsh JA et al (1995) Gender comparisons in human lung cancer: analysis of p53 mutations, anti-p53 serum antibodies and C-erbB-2 expression. Carcinogenesis 16(5):993–1002. doi:10.1093/carcin/16.5.993

24. Shi SR, Key ME, Kalra KL (1991) Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 39(6):741–748

25. Hsu SM, Raine L, Fanger H (1981) Use of avidin-biotin-peroxidase complex (ABC) in immunoperoxidase techniques: a comparison between ABC and unlabeled antibody (PAP) procedures. J Histochem Cytochem 29(4):577–580

26. Hosmer DW, Lemeshow S (1989) Applied logistic regression. Wiley, New York

27. Crew KD, Neugut AI (2004) Epidemiology of upper gastrointestinal malignancies. Semin Oncol 31(4):450–464. doi:10.1053/j.seminoncol.2004.04.021

28. Saeki H, Ohno S, Miyazaki M, Araki K, Egashira A, Kawaguchi H et al (2002) p53 protein accumulation in multiple oesophageal squamous cell carcinoma: relationship to risk factors. Oncology 62(2):175–179. doi:10.1159/000048264

29. Montesano R, Hollstein M, Hainaut P (1996) Genetic alterations in esophageal cancer and their relevance to etiology and pathogenesis: a review. Int J Cancer 69(3):225–235. doi:10.1002/(SICI)1097-0215(19960621)69:3<225::AID-IJC13>3.0.CO;2-6

30. Vaughan TL, Kristal AR, Blount PL, Levine DS, Galipeau PC, Prevo LJ et al (2002) Nonsteroidal anti-inflammatory drug use, body mass index, and anthropometry in relation to genetic and flow cytometric abnormalities in Barrett's esophagus. Cancer Epidemiol Biomarkers Prev 11(8):745–752

31. Palli D, Caporaso NE, Shiao YH, Saieva C, Amorosi A, Masala G et al (1997) Diet, Helicobacter pylori, and p53 mutations in gastric cancer: a molecular epidemiology study in Italy. Cancer Epidemiol Biomarkers Prev 6(12):1065–1069

32. Theisen J, Peters JH, Fein M, Hughes M, Hagen JA, Demeester SR et al (2005) The mutagenic potential of duodenoesophageal reflux. Ann Surg 241(1):63–68

33. Bian YS, Osterheld MC, Bosman FT, Benhattar J, Fontolliet C (2001) p53 gene mutation and protein accumulation during neoplastic progression in Barrett's esophagus. Mod Pathol 14(5):397–403. doi:10.1038/modpathol.3880324

34. McManus DT, Olaru A, Meltzer SJ (2004) Biomarkers of esophageal adenocarcinoma and Barrett's esophagus. Cancer Res 64(5):1561–1569. doi:10.1158/0008-5472.CAN-03-2438

35. Trivers KF, De Roos AJ, Gammon MD, Vaughan TL, Risch HA, Olshan AF et al (2005) Demographic and lifestyle predictors of survival in patients with esophageal or gastric cancers. Clin Gastroenterol Hepatol 3(3):225–230. doi:10.1016/S1542-3565(04)00613-5