

Single nucleotide polymorphisms of 8 inflammation-related genes and their associations with smoking-related cancers

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Tobacco smoke and its metabolites are carcinogens that increase tissue oxidative stress and induce target tissue inflammation. We hypothesized that genetic variation of inflammatory pathway genes plays a role in tobacco-related carcinogenesis and is modified by tobacco smoking. We evaluated the association of 12 single nucleotide polymorphisms of 8 inflammation-related genes with tobacco-related cancers (lung, oropharynx, larynx, esophagus, stomach, liver, bladder, and kidney) using 3 case-control studies from: Los Angeles (population-based; 611 lung and 553 upper aero-digestive tract cancer cases and 1,040 controls), Taixing, China (population-based; 218 esophagus, 206 stomach, 204 liver cancer cases, and 415

Key words: *IL10*, *TNF*, single nucleotide polymorphisms, inflammation, tobacco-related cancer

Abbreviations aOR: adjusted odds ratio; aROR: adjusted ratio of odds ratios; BFDP: Bayesian false discovery probability; CI: confidence interval; cOR: crude odds ratio; FPRP: false positive report probability; HWE: Hardy-Weinberg equilibrium; LA: Los Angeles; MAF: minor allele frequency; MSKCC: Memorial Sloan-Kettering Cancer Center; NSCLC: non-small cell lung cancer; OR: odds ratio; ROR: ratio of odds ratios; SCC: squamous cell carcinoma; SNP: single nucleotide polymorphism; sOR: summary odds ratio; UADT: upper aero-digestive tract

Additional Supporting Information may be found in the online version of this article.

Grant sponsor: NIH National Institute of Environmental Health Sciences (National Cancer Institute, Department of Health and Human Services); **Grant numbers:** ES06718, ES01167, DA11386, CA90833, CA77954, CA09142, CA96134, CA128099, GM53275; **Grant sponsors:** Alper Research Center for Environmental Genomics, The Seymour Family Gift for Innovative Investigator-Initiated Research in Bladder Cancer (UCLA Jonsson Comprehensive Cancer Center), UCLA Center for Occupational and Environmental Health

DOI: 10.1002/ijc.25214

History: Received 16 Nov 2009; Accepted 18 Jan 2010; Online 28 Jan 2010

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controls), and Memorial Sloan-Kettering Cancer Center (hospital-based; 227 bladder cancer cases and 211 controls). After adjusting for age, education, ethnicity, gender, and tobacco smoking, *IL10* rs1800871 was inversely associated with oropharyngeal cancer (CT+TT vs. CC adjusted odds ratio [aOR]: 0.69, 95% confidence interval [CI]: 0.50–0.95), and was positively associated with lung cancer among never smokers (TT vs. CT+CC aOR: 2.5, 95% CI: 1.3–5.1) and inversely with oropharyngeal cancer among ever smokers (CT+TT vs. CC aOR: 0.63, 95% CI: 0.41–0.95). Among all pooled never smokers (588 cases and 816 controls), *TNF* rs1799964 was inversely associated with smoking-related cancer (CC vs. CT+TT aOR: 0.36, 95% CI: 0.17–0.77). Bayesian correction for multiple comparisons suggests that chance is unlikely to explain our findings (although epigenetic mechanisms may be in effect), which support our hypotheses, suggesting that *IL10* rs1800871 is a susceptibility marker for oropharyngeal and lung cancers, and that *TNF* rs1799964 is associated with smoking-related cancers among never smokers.

Tobacco smoking is a major risk factor for malignancies of the lung, upper aero-digestive tract (UADT), stomach, pancreas, liver, kidney, urinary tract, uterine cervix, and bone marrow. Epithelial cells in many of these organs are repeatedly exposed to components and metabolites of tobacco smoke, which are carcinogenic and potent inducers of inflammation. The high concentration of free radicals contained in and generated by tobacco smoke can lead to cancer through oxidative DNA damage mediated by inflammation-associated production of reactive oxygen species.

Chronic inflammation, characterized in part by altered cytokine levels, is believed to play a role in tumor initiation and promotion. Inflammatory conditions such as ulcerative colitis and inflammatory bowel disease have well-established associations with colorectal cancer,¹ and lung cancer has been connected to inflammatory diseases such as tuberculosis and pneumonia.^{2,3} Additionally, cancers of the stomach, liver, and esophagus have been attributed to chronic inflammation as a result of persistent infection.⁴ An individual's cancer risk may be affected by genetic variations in essential cell regulatory pathways^{5,6} and inflammatory responses,^{7,8} and tobacco smoking may modify these effects,^{9,10} suggesting that genetic variation may be important susceptibility markers for tobacco-related cancers.

In the current study, we use a pathway-based approach using data from 3 case-control studies (Los Angeles [LA] County, Taixing, China, and Memorial Sloan-Kettering Cancer Center [MSKCC]) to test the hypotheses that single nucleotide polymorphisms (SNPs) in inflammation-related genes are associated with smoking-related cancers of the lung, oropharynx, larynx, esophagus, stomach, liver, bladder, and kidney, and that their effects are modified by tobacco smoking.

Material and Methods

Study design and participants

Detailed descriptions of the 3 case-control studies reported in this manuscript have been published for the LA,¹¹ Taixing,¹² and MSKCC¹³ and studies, which are briefly described later. All study participants provided written consent, and study protocols were approved by appropriate review boards. Sub-

jects not meeting study-specific inclusion criteria were excluded from enrollment.

The LA study was population-based, consisting of histologically confirmed incident lung ($n = 611$) and UADT ($n = 553$) cancer cases obtained from the LA County cancer registry administered through the Cancer Surveillance Program at the University of Southern California; 1,040 controls without a history of lung or UADT cancer were matched on gender, age, and residential neighborhood, using an algorithm to identify eligible controls from a census of each case's neighborhood. To be eligible, subjects had to be (i) 18–65 years of age during 1999–2004, (ii) a resident of LA County at time of diagnosis (cases) or recruitment (controls), and (iii) able to speak English or Spanish, or have a translator available at home. Recruitment rates among eligible cases were 39 and 46% for lung and UADT cases, respectively, and 79% for controls. Buccal cell samples were obtained at the end of interviews for DNA analysis.

The Taixing study was also population-based and conducted in 2000. Newly diagnosed and pathologically or clinically confirmed cases of stomach ($n = 206$), liver ($n = 204$), and esophageal ($n = 218$) cancers were obtained from the Taixing Tumor Registry operated through the Taixing Center for Disease Control and Prevention. A common group of healthy controls from the general population registry ($n = 415$) was frequency matched by age, gender, and village. Eligibility criteria required that all subjects were (i) at least 20 years old, (ii) in stable medical condition, and (iii) living in Taixing for at least 10 years. Recruitment rates were 67, 65, and 57% for eligible esophagus, stomach, and liver cancer cases, respectively, and 89% for controls. Blood samples were collected at the end of interviews for DNA analysis.

The MSKCC study, conducted during 1993–1997, was hospital-based and consisted of 227 pathologically confirmed bladder cancer cases sampled from the MSKCC who had recently been diagnosed or undergone bladder surgery. Two-hundred and eleven controls who had resided in the United States for at least a year were recruited from the MSKCC blood bank or from MSKCC patients who did not have cancer diagnoses and were in stable medical condition. Ninety-five percent of cases and 92% of controls agreed to participate; blood samples were collected at the end of interviews for DNA analysis.

Standardized questionnaires appropriate for each of the 3 studies were administered in person by trained staff. Data collected across all 3 studies included demographic information; detailed behavioral factors such as diet, alcohol use, and exposure to tobacco smoke; other environmental and occupational exposures; personal and family medical histories; and other exposures considered known or possible risk factors for cancers specific to each study. Between-study variation among the common demographic variables was greatest for race/ethnicity, which was most heterogeneous in the LA study (59.3% White, 17.0% Hispanic, 11.9% African-American, 8.7% Asian-American, and 3.0% other) and least in the Taixing study (100% Chinese); less than 5% of MSKCC study participants were non-White.

SNP selection and analysis

We focused on functional and potentially functional SNPs (such as amino acid-changing polymorphisms) and SNPs located in regions regulating gene transcription (such as promoter areas). We decided a priori to exclude SNPs from analysis that did not meet the following criteria among the study-specific control groups: (i) minor allele frequency (MAF) $\geq 5\%$, (ii) Hardy-Weinberg equilibrium (HWE) p -value $>$ Bonferroni-adjusted p -value, and (iii) genotyping call rate $\geq 80\%$ for SNPlex and $\geq 95\%$ for TaqMan.

The majority of SNPs that violated HWE in our initial pool of SNPs were excluded from analysis due to low genotyping rates or minor allele frequencies. After using a Bonferroni-adjusted cut-point among the study-specific control groups to assess deviations from HWE, 1 SNP was excluded from the LA study (*TNF* rs1799724 HWE p value $<$ 0.0001), another SNP was dropped from the Taixing study (*IL13* rs20541 HWE p value $<$ 0.0001), and 1 more from the MSKCC study (*IL13* rs20541 HWE p value = 0.00018). The final pool of SNPs included in our study had allele frequencies that were in the expected range of normal variation, and none had HWE $p <$ 0.05 among the study-specific control groups. Six SNPs met these criteria across all 3 studies (*IL10* rs1800871, *TNF* rs1799964, *TNF* rs1800629, *LTA* rs909253, *IFNGR1* rs11914, and *IFNG* rs2069705), whereas another 6 met inclusion criteria for at least 1 of the studies (*IL10* rs1800872, *IL10* rs1800896, *IL1A* rs17561, *IL1B* rs1143627, *IL1B* rs16944, and *IL6* rs1800796). These 12 SNPs and details of their inclusion criteria are reported in Supporting Information Table S1. Because population substructure can affect a SNP's distribution, we also examined HWE by ethnicity in the LA study. At an alpha level of 0.05, deviation from HWE was suggested for several SNPs (*IL1B* rs1143627 among Hispanics, *IL10* rs1800871 in Asian-Americans, and *LTA* rs909253 for African-Americans), but none exceeded the Bonferroni-adjusted cut-point of $0.05/12 = 0.0042$. In instances where 2 or more SNPs are in strong linkage disequilibrium (*i.e.*, $r^2 \geq 0.9$), the SNP with the more reliable signal is presented. Although 3 SNPs in the *IL10* promoter region were within 0.5 kb of each other (rs1800896, rs1800871, and

rs1800872), haplotype analysis was not conducted as rs1800896 was not genotyped in the LA and MSKCC studies, and because initial analysis suggested a block size of only 2 SNPs in the Taixing study.

Laboratory analysis

DNA was isolated using a modified phenol-chloroform method. SNPs were genotyped using the SNPlex assay (Applied Biosystems [ABI], Foster City, CA); 2 were also genotyped with ABI's TaqMan assay (*IL10* rs1800871 and *IFNGR1* rs11914). Briefly, DNA aliquots from cases and controls were randomized onto PCR plates with the appropriate reaction mix. For SNPlex reactions, allele-specific oligonucleotide probes were hybridized to target genomic sequences. The hybridization products were purified of excess probe, amplified by PCR, and captured in streptavidin-coated microtiter plates. Fluorescently labeled ZipChute probes were then hybridized to the streptavidin-bound amplicons and detected by capillary electrophoresis. In the TaqMan assay, fluorescently labeled sequence-specific primers were used in PCR reactions in a total volume of 5 μ l with the following modified protocol: denaturation at 92°C for 10 min followed by 60 cycles at 92°C for 15 sec and extension at 62°C for 80 sec. Genotype detection for SNPlex and TaqMan assays was performed using an ABI 3730 DNA Analyzer with ABI Genemapper 4.0 software and an ABI 7900 machine with SDS 2.3 software, respectively. Call rates were $>85\%$ for SNPlex and $>97\%$ for TaqMan. Reproducibility was 0.978 for the SNPlex assay (based on resequencing a 3% random sample), and 0.997 for the TaqMan assay (using a 5% random sample). Concordance for SNPs genotyped on both platforms was 0.943 for *IL10* rs1800871 and 0.996 for *IFNGR1* rs11914.

Statistical analysis

Fisher's exact test was used to assess deviation from HWE, and all data were analyzed with SAS version 9.1.3. The odds ratio (OR) and 95% confidence interval (CI) for each SNP-cancer association of each study and within smoking strata were estimated using unconditional logistic regression. To test the hypothesis that an increasing number of risk alleles is associated with cancer (*i.e.*, monotonic-response model), we tested for linear trend of the odds ratio by treating the number of risk alleles as a continuous variable in logistic regression models, designating the homozygous ancestral genotype as the reference group based on SNP-specific information at the National Center for Biotechnology Information's dbSNP database.¹⁴ We used these cancer site-specific genotype-cancer associations for each SNP to determine the appropriateness of selecting an inheritance model (*e.g.*, dominant, recessive). Covariates in the LA study included race/ethnicity (non-Hispanic White, Hispanic, African-American, Asian-American, and other), pack-years of tobacco smoking (continuous), drink-years of alcohol consumption for UADT cancers (continuous), and years of education (continuous).

To minimize confounding, age was divided into fine categories (29–34, 35–36, 37–38, 39–40, 41–42, 43–44, 45–46, 47–48, 49–50, 51–52, 53–54, 55–56, 57–58, and 59–62), and controls who were more than 3 years older than the oldest case or more than 3 years younger than the youngest case were excluded. This resulted in the exclusion of 11 controls from lung cancer analyses; no controls were excluded from UADT analyses. Analyses in the Taixing study were adjusted for gender, alcohol drinking (4-level ordinal), education (4-level ordinal), age (continuous), pack-years (continuous), hepatitis B surface antigen for liver cancer, and *Helicobacter pylori* infection status for stomach cancer. Regression models in the MSKCC study included race (White vs. non-White), gender, smoking status (ever vs. never), age (continuous), and years of education (continuous). Stratified analyses by smoking status (ever and never smokers) were limited to instances for which there were at least 75 cases to ensure adequate precision, and pack-years of smoking was included in regression models to address residual confounding among ever smokers. We tested the effects of the SNPs and tobacco smoking for departure from multiplicativity by fitting a model with smoking (ever/never), genotype, and their product term, and calculating the ratio of odds ratios (ROR) by taking the natural antilog of the estimated coefficient of the product term. Potential confounders, including ethnicity, age, gender, and pack-years of smoking were included in logistic regression models, and individuals with the nonrisk genotype who had never smoked were designated as the reference group.

For SNPs genotyped across all 3 studies that had point estimates consistent in direction and magnitude over all cancer sites, we investigated whether those SNPs were associated with smoking-related cancer by pooling all cases and controls across all 3 studies and the different tumor sites. We adjusted for study location, gender, race/ethnicity, age, and tobacco smoking (ever vs. never). Because race/ethnicity is highly correlated with study location, we tested for the presence of multicollinearity by assessing the variance inflation factor and by testing for heterogeneity between models with and without terms for ethnicity. Neither method suggested that multicollinearity was an important factor, so categories for race/ethnicity and study location were combined into 1 variable and included as a new covariate in adjusted logistic regression analyses for pooled cancer sites.

Given the number of comparisons made in this study, we addressed the possibility of chance findings using 2 Bayesian approaches. The false positive report probability (FPRP) facilitates identifying noteworthy observations when the probability of a false positive is below an investigator-predetermined threshold.¹⁵ The Bayesian false discovery probability (BFDP) is a progression of FPRP and considers the ratio of the cost of missing a true association to the cost of a false discovery.¹⁶ In both methods, an observation from our study was viewed in light of prior knowledge (*i.e.*, prior probability) to assess the posterior probability that the association is not null. The value of the prior probability is subjective and guided by epi-

demologic data and existing knowledge of the gene and related SNPs. We considered prior probabilities ranging from 0.10 to 0.01, a main effect OR of 1.5 (or 0.67), a smoking-stratified OR of 2.5 (or 0.40), and a FPRP cut-point of 0.4 and a BFDP threshold of 0.8 to determine noteworthy findings.

Results

Characteristics of our study population of 2,049 smoking-related cancer cases and 1,666 controls are presented in Table 1. When compared with the LA and MSKCC studies, the majority of Taixing participants had completed less than 12 years of education and was less likely to have reported ever drinking alcohol. Controls were noticeably younger than cases in the MSKCC study. The estimated effects of the 12 SNPs stratified by cancer site are reported in Supporting Information Tables S2 and S3; selected results are summarized in Figure 1a. Oropharyngeal cancer was inversely associated with *IL10* rs1800871 among CT heterozygotes (adjusted OR [aOR]: 0.67, 95% CI: 0.48–0.94) and possibly among TT homozygotes (aOR: 0.78, 95% CI: 0.44–1.4), suggesting a dominant inheritance model (aOR: 0.69, 95% CI: 0.50–0.95). An inverse association was also observed between *IFNG* rs2069705 and oropharyngeal cancer (CT and TT versus CC aOR: 0.72, 95% CI: 0.52–1.0) (Supporting Information Table S2). When compared with *IL1B* rs16944 GG homozygotes, the A allele was more common among lung cancer cases (aOR: 1.3, 95% CI: 1.0–1.8). This association, which was not modified by smoking status, appeared to be consistent across ethnicity.

Because tobacco smoking is a strong risk factor for our outcomes, we re-examined the SNP-cancer associations, stratifying by ever/never smoking status. These results are reported in Supporting Information Table S4 and summarized in Figure 1a. Lung cancer was associated with *IL10* rs1800871 among never smokers (TT versus CC or CT aOR: 2.5, 95% CI: 1.3–5.1) but not among ever smokers, though there was some heterogeneity across ethnicity. Although the point estimate for Whites was less than 1.0, estimates were greater than 1.0 for the other ethnicities, though they tended to be imprecise. Oropharyngeal cancer was inversely associated with *IL10* rs1800871 among ever smokers (CT or TT versus CC aOR: 0.63, 95% CI: 0.41–0.95) and positively associated with *IFNG* rs2069705 among never smokers (CC versus CT or TT aOR: 1.9, 95% CI: 1.0–3.5). Esophageal cancer in the Taixing study was less common among ever smokers who had at least 1 variant G allele for *IFNGR1* rs11914 (aOR: 0.38, 95% CI: 0.18–0.80). Risk estimates for SNP-cancer associations after restricting to current smokers were in the same direction as smoking stratified results but greater in magnitude (*i.e.*, further from the null) and less precise.

Of the 6 SNPs that were genotyped across all 3 studies, the most consistent SNP-cancer estimates were observed for *TNF* rs1799964 among never smokers. Smoking-related cancers were much less common among never smokers

Table 1. Demographic and behavioral characteristics of cancer cases and controls, by study location

	All studies pooled				Los Angeles				Taixing				MSKCC											
	Case ¹		Control		Lung		UADT		Control		Stomach		Liver		Bladder		Control							
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%						
Age	N = 1,040																							
	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%						
35<	49	2.4	133	8.0	4	0.7	25	4.5	51	4.9	1	0.5	16	7.8	19	4.6	2	0.9	63	30.0				
35-44	185	9.0	278	16.7	57	9.3	65	11.8	171	16.4	5	2.3	12	5.8	37	18.1	41	9.9	6	2.6	66	31.3		
45-54	760	37.1	641	38.5	301	49.3	249	45.0	499	48.0	66	30.3	38	18.5	63	30.9	98	23.6	35	15.4	44	20.9		
55+	1,054	51.4	614	36.9	249	40.8	214	38.7	319	30.7	145	66.5	155	75.2	88	43.1	257	61.9	184	81.1	38	18.0		
Missing	1	0.1	0	0	0	0	0	0	0	0	1	0.5	0	0	0	0	0	0	0	0	0	0		
Gender																								
Male	1,371	66.9	1,062	63.7	303	49.6	420	75.9	623	59.9	141	64.7	138	67.0	159	77.9	287	69.2	189	83.3	152	72.0		
Female	678	33.1	591	35.5	308	50.4	133	24.1	417	40.1	77	35.3	68	33.0	45	22.1	128	30.8	38	16.7	46	21.8		
Missing	0	0	13	0.8	0	0	0	0	0	0	0	0.0	0	0	0	0	0	0	0	0	13	6.2		
Ethnicity																								
White	920	44.9	825	49.5	359	58.8	331	59.9	634	61.0											206	90.8	191	90.5
Hispanic	176	8.6	206	12.4	70	11.5	100	18.1	204	19.6											6	2.6	2	1.0
African-American	174	8.5	104	6.2	96	15.7	66	11.9	102	9.8											7	3.1	2	1.0
Asian-American	113	5.5	63	3.8	70	11.5	41	7.4	62	6.0											1	0.4	1	0.5
Other	30	1.5	38	2.3	15	2.5	13	2.4	37	3.6											2	0.9	1	0.5
Han Chinese	628	30.7	415	24.9							218	100	206	100	204	100	415	100						
Missing	8	0.4	15	0.9	1	0.2	2	0.4	1	0.1	0	0	0	0	0	0	0	0	0	0	5	2.2	14	6.6
Education (year)																								
<12	851	41.5	458	27.5	107	17.5	115	20.8	116	11.2	212	97.2	203	98.5	191	93.6	339	81.7	19	8.4	3	1.4		
12 or more	1,195	58.3	1,207	72.4	504	82.5	438	79.2	923	88.8	3	1.4	3	1.5	13	6.4	76	18.3	208	91.6	208	98.6		
Missing	3	0.1	1	0.1	0	0	0	0	1	0.1	3	1.4	0	0	0	0	0	0	0	0	0	0		
Tobacco (pack-years)																								
Never smoker	588	28.7	816	49.0	110	18.0	159	28.8	492	47.3	94	43.1	92	44.7	85	41.7	217	52.3	38	16.7	107	50.7		
>0-20	416	20.3	493	29.6	102	16.7	132	23.9	353	33.9	45	20.6	48	23.3	58	28.4	91	21.9	29	12.8	49	23.2		
>20-40	507	24.7	236	14.2	202	33.1	139	25.1	136	13.1	48	22.0	41	19.9	38	18.6	83	20.0	31	13.7	17	8.1		
>40	487	23.8	94	5.6	197	32.2	123	22.2	58	5.6	24	11.0	20	9.7	11	5.4	23	5.5	107	47.1	13	6.2		
Missing	51	2.5	27	1.6	0	0	0	0	1	0.1	7	3.2	5	2.4	12	5.9	1	0.2	22	9.7	25	11.9		

Table 1. Demographic and behavioral characteristics of cancer cases and controls, by study location (Continued).

	All studies pooled																					
	Los Angeles						Taixing			MSKCC												
	Case ¹	Control	Lung	UADT	Control	Esophagus	Stomach	Liver	Control	Bladder	Control											
	N = 2,049	N = 1,666	N = 611	N = 553	N = 1,040	N = 218	N = 206	N = 204	N = 415	N = 227	N = 211											
	%	%	%	%	%	%	%	%	%	%	%											
Alcohol																						
Never	618	30.2	507	30.4	170	27.8	94	17.0	264	25.4	116	53.2	111	53.9	87	42.7	207	49.9	31	13.7	36	17.1
Ever	1,395	68.1	1,138	68.3	440	72.0	457	82.6	772	74.2	95	43.6	90	43.7	105	51.5	205	49.4	187	82.4	161	76.3
Missing	36	1.8	21	1.3	1	0.2	2	0.4	4	0.4	7	3.2	5	2.4	12	5.9	3	0.7	9	4.0	14	6.6
<i>H. pylori</i> infection																						
Chronic hepatitis B																						

¹Also includes 30 kidney cancer cases from MSKCC.

Abbreviations: MSKCC: Memorial Sloan-Kettering Cancer Center; UADT: upper aero-digestive tract.

homozygous for the variant *TNF* rs1799964 C allele compared with never smokers with at least 1 T allele (aOR: 0.36, 95% CI: 0.17–0.77). This inverse association was not observed among ever smokers (Fig. 1b and Supporting Information Table S5). When stratified by ethnicity, the relative rarity of cancers among never smokers compared with ever smokers tended to persist, although smoking-stratified estimates for some ethnicities were imprecise.

The estimated effects of genotype-smoking-status combinations for selected SNPs are shown in Table 2. In site-stratified analysis, we observed that the estimated joint effect of recessive *IL10* rs1800871 genotype and tobacco smoking was less than what would be expected under the null hypothesis of multiplicativity of effects among lung cancer cases and controls (aROR: 0.31, 95% CI: 0.14–0.69), which persisted across ethnicity, although adjusting for pack-years attenuated the estimate. There was also an indication of departure from multiplicative effects for *IFNGR1* rs11914 and esophageal cancer in the Taixing study (aROR: 0.37, 95% CI: 0.13–1.0). In our pooled analysis, there was evidence of greater than multiplicative interaction among individuals recessive for *TNF* rs1799964 (aROR: 3.4, 95% CI: 1.4–8.6), which was fairly consistent across ethnicity with the exception of African-Americans, whose unstable estimates were a reflection of that group having the least number of individuals homozygous for the rare genotype among controls.

Given the many comparisons in our study, we considered the probability of chance finding (Supporting Information Table S6). Assuming a prior probability of 0.10 and a FPRP cut-point of 0.4, the associations between lung cancer and *IL1B* rs16944 and between oropharyngeal cancer and polymorphisms of *IL10* rs1800871 and *IFNG* rs2069705 appear to be noteworthy. Under the same prior, and assuming that false nondiscovery is 4 times as costly as false discovery (*i.e.*, a BFDP threshold of 0.8), the association between oropharyngeal cancer and *IL10* rs1800871 may be important. Considering associations that are below both FPRP and BFDP thresholds, *IL10* rs1800871 appears to be an important marker for oropharyngeal cancer risk. *IL10* rs1800871 may also be associated with lung cancer among never smokers and with oropharyngeal cancer among ever smokers. Chance does not seem to be a likely explanation in our study population. The *TNF* rs1799964 SNP, which only appeared to be associated with stomach cancer in site-specific analyses, also seems to be associated with smoking-related cancers among never smokers, even at a 5% prior probability. Additional associations adjusted for multiple comparisons may be noteworthy for several other smoking-stratified associations at a prior probability of 0.10 but none passed FPRP and BFDP criteria using more conservative priors.

Discussion

Among never smokers in our overall study population of 2,049 cases and 1,666 controls, we found that *TNF* rs1799964 was associated with smoking-related cancer. Cancer site-

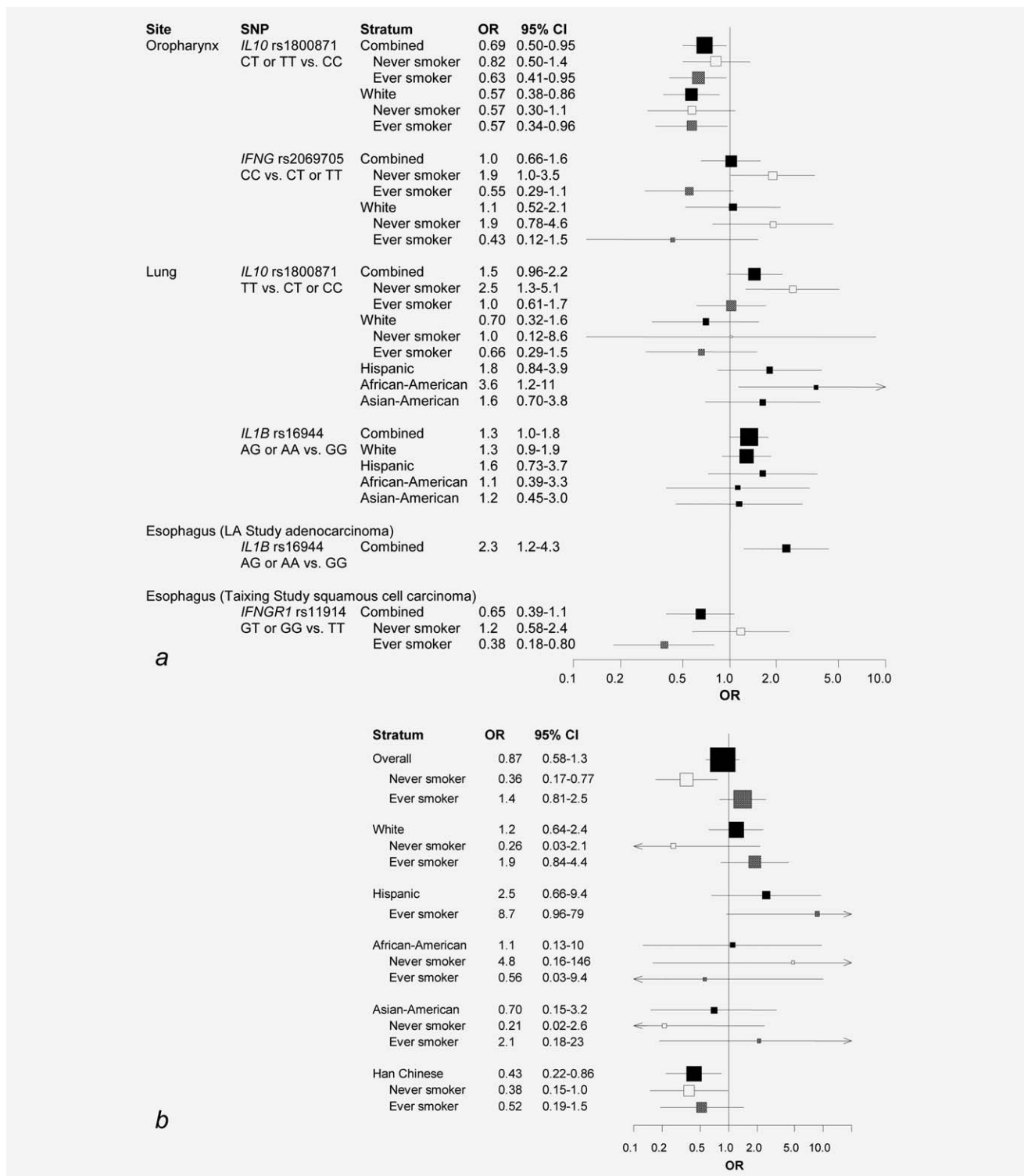


Figure 1. (a) Selected single nucleotide polymorphism-cancer associations (black boxes) stratified by cancer site and smoking status (white and hatched boxes represent never and ever smokers, respectively). Odds ratios (OR) and 95% confidence intervals (CI) adjusted for age, gender, education, tobacco smoking, ethnicity, and alcohol use (oropharynx and esophagus). (b) *TNF* rs1799964-cancer associations (black boxes) among pooled cancer cases and controls stratified by ethnicity and smoking status (white and hatched boxes represent never and ever smokers, respectively). Odds ratios (OR) and 95% confidence intervals (CI) adjusted for study location, age, gender, education, tobacco smoking, and ethnicity.

Table 2. Modification of selected SNP-specific odds ratios for tobacco-related cancers pooled and stratified by ethnicity, smoking status, and cancer site

SNP and cancer site	Ever smoker	Genotype	Case	Control	cOR	95% CI	aOR	95% CI	FPRP prior probability		
									0.10	0.05	0.01
<i>TNF rs1799964</i>											
All cancer sites pooled	No	TT + CT	434	633	1.0		1.0				
	No	CC	10	35	0.42	0.20–0.85	0.41	0.19–0.85	0.22	0.37	0.76
	Yes	TT + CT	1,044	650	2.3	2.0–2.7	2.4	2.0–2.8	<0.01	<0.01	<0.01
	Yes	CC	45	20	3.3	1.9–5.6	3.3	1.9–5.8	<0.01	<0.01	0.02
				ROR	3.4	1.4–8.2	3.4	1.4–8.6	0.25	0.41	0.78
White	No	TT + CT	111	329	1.0		1.0				
	No	CC	1	12	0.25	0.03–1.9	0.26	0.03–2.1			
	Yes	TT + CT	528	314	5.0	3.9–6.4	3.6	2.8–4.8	<0.01	<0.01	<0.01
	Yes	CC	25	9	8.2	3.73–18	6.7	2.9–15	0.01	0.01	0.07
				ROR	6.7	0.74–60	7.1	0.76–66			
Hispanic	No	TT + CT	43	75	1.0		1.0				
	No	CC	0	3							
	Yes	TT + CT	77	102	1.3	0.82–2.1	1.3	0.80–2.2			
	Yes	CC	6	1	10	1.2–90	11	1.3–97	0.75	0.87	0.87
				ROR							
African-American	No	TT + CT	19	29	1.0		1.0				
	No	CC	1	1	1.5	0.11–32	2.7	0.15–49			
	Yes	TT + CT	103	43	3.7	1.9–7.2	3.5	1.8–7.1	0.02	0.05	0.20
	Yes	CC	1	1	1.5	0.09–26	1.8	0.10–31			
				ROR	0.28	0.01–15	0.18	0.004–11			
Asian-American	No	TT + CT	36	26	1.0		1.0				
	No	CC	1	3	0.23	0.02–2.4	0.24	0.02–2.5			
	Yes	TT + CT	38	21	1.3	0.60–2.6	1.3	0.63–2.7			
	Yes	CC	3	1	2.1	0.20–22	2.2	0.21–22			
				ROR	6.8	0.25–181	10.0	0.31–332			
Han Chinese	No	TT + CT	221	164	1.0		1.0				
	No	CC	7	14	0.37	0.15–0.94	0.38	0.14–0.98	0.47	0.65	0.91
	Yes	TT + CT	283	151	1.4	1.1–1.8	1.5	1.1–2.2	0.18	0.31	0.70
	Yes	CC	8	8	0.74	0.27–2.0	0.78	0.27–2.2			
				ROR	1.4	0.37–5.6	1.4	0.33–5.4			
<i>IL10 rs1800871</i>											
Lung	No	CC + CT	63	387	1.0		1.0				
	No	TT	28	33	5.2	3.0–9.2	3.1	1.6–5.9	0.02	0.04	0.19
	Yes	CC + CT	387	424	5.6	4.2–7.6	1.2	0.82–1.8			
	Yes	TT	47	61	4.7	3.0–7.5	1.2	0.68–2.1			
				ROR	0.16	0.08–0.33	0.31	0.14–0.69	0.12	0.23	0.60
White	No	CC + CT	24	263	1.0		1.0				
	No	TT	1	10	1.1	0.13–8.9	1.0	0.12–8.3			
	Yes	CC + CT	270	266	11	7.1–17	1.8	1.0–3.2	0.29	0.46	0.82
	Yes	TT	15	22	7.5	3.4–16	1.2	0.45–3.2			
				ROR	0.61	0.07–5.6	0.67	0.07–6.5			

Table 2. Modification of selected SNP-specific odds ratios for tobacco-related cancers pooled and stratified by ethnicity, smoking status, and cancer site (Continued).

SNP and cancer site	Ever smoker	Genotype	Case	Control	cOR	95% CI	aOR	95% CI	FPRP prior probability		
									0.10	0.05	0.01
Hispanic	No	CC + CT	19	71	1.0		1.0				
	No	TT	9	10	3.4	1.2–9.5	3.8	1.2–12	0.43	0.61	0.89
	Yes	CC + CT	30	87	1.3	0.67–2.5	0.72	0.31–1.7			
	Yes	TT	5	20	0.93	0.31–2.8	0.70	0.21–2.3			
					ROR	0.21	0.05–0.93	0.25	0.05–1.2		
African-American	No	CC + CT	8	28	1.0		1.0				
	No	TT	0	1							
	Yes	CC + CT	58	42	4.8	2.0–12	1.1	0.33–3.8			
	Yes	TT	17	6	9.9	2.9–34	4.7	1.1–21	0.65	0.80	0.95
					ROR						
Asian-American	No	CC + CT	9	16	1.0		1.0				
	No	TT	18	11	2.9	0.96–8.8	3.7	1.1–12	0.52	0.70	0.92
	Yes	CC + CT	18	10	3.2	1.0–10	0.76	0.15–3.9			
	Yes	TT	10	12	1.5	0.46–4.8	0.48	0.10–2.4			
					ROR	0.17	0.03–0.84	0.18	0.03–1.1		
<i>IFNG</i> rs2069705											
Oropharynx	No	TT + CT	60	339	1.0		1.0				
	No	CC	21	67	1.8	1.0–3.1	1.9	1.0–3.4	0.30	0.47	0.82
	Yes	TT + CT	115	360	1.8	1.3–2.6	0.82	0.52–1.3			
	Yes	CC	16	90	1.0	0.55–1.8	0.48	0.24–0.98	0.36	0.55	0.86
					ROR	0.31	0.14–0.70	0.31	0.13–0.73	0.19	0.33
White	No	TT + CT	44	233	1.0		1.0				
	No	CC	8	21	2.0	0.84–4.8	2.0	0.83–5.0			
	Yes	TT + CT	79	239	1.8	1.2–2.6	0.90	0.51–1.6			
	Yes	CC	3	25	0.64	0.18–2.2	0.39	0.11–1.4			
					ROR	0.18	0.04–0.81	0.21	0.05–1.0	0.68	0.82
<i>IFNGR1</i> rs11914											
Esophagus (Taixing)	No	TT	67	161	1.0		1.0				
	No	GT + GG	17	36	1.1	0.60–2.2	1.1	0.55–2.2			
	Yes	TT	96	141	1.6	1.1–2.4	2.0	0.97–4.0			
	Yes	GT + GG	12	42	0.69	0.34–1.4	0.74	0.29–1.9			
					ROR	0.40	0.15–1.0	0.37	0.13–1.0	0.51	0.68

Abbreviations: aOR: odds ratio (OR) adjusted for study location/ethnicity (pooled analysis only), ethnicity (lung analysis only), age, gender, education, tobacco smoking, and alcohol use (oropharynx and esophagus analyses only); CI: confidence interval; cOR: crude OR; FPRP: false positive report probability. FPRP values are the posterior probabilities of reporting a false positive result, given the statistical power to detect a smoking-stratified aOR or ROR of at least 2.5. Values of FPRP less than 0.4 are bold-faced, indicating noteworthy observations; ROR: ratio of odds ratios; SNP: single nucleotide polymorphism.

specific analysis suggests that oropharyngeal cancer is inversely associated with *IL10* rs1800871 and *IFNG* rs2069705. Smoking-stratified analyses suggest that some SNP-cancer associations become more apparent within strata of smoking status and that some associations may be site-specific. *TNF* rs1799964, for example, seems to be an important SNP among never smokers for smoking-related cancer as a whole, while the *IL10* rs1800871 association with lung cancer was

observed only among never smokers (aOR: 2.5, 95% CI: 1.3–5.1 vs. aOR: 1.0, 95% CI: 0.61–1.7 for ever smokers). After adjustment for multiple comparisons, the associations between *TNF* rs1799964 and any smoking-related cancer among never smokers and between *IL10* rs1800871 and cancers of the oropharynx and lung do not appear to be due to chance.

Whether *IL10* polymorphisms affect lung cancer risk remains to be determined but some reports suggest an

association, although differences in models of inheritance and risk estimates suggest some heterogeneity. A population-based case-cohort study estimated a 60% increase in lung cancer risk for individuals with at least 1 copy of the *IL10* rs1800872 variant A allele (which is in high linkage disequilibrium with rs1800871), and a slightly weaker association among current smokers.¹⁷ A positive association between non-small cell lung cancer (NSCLC) and *IL10* rs1800871 was estimated by Van Dyke *et al.* in a population-based case-control study among Caucasian women (aOR: 1.39, 95% CI: 0.96–2.02), and the magnitude of the association was still elevated, though less precisely, among a smaller number of African-American women (aOR: 1.32, 95% CI: 0.60–2.88).¹⁸ *IL10* rs1800871 was recessively associated with NSCLC in a Chinese population (OR: 1.37, 95% CI: 1.10–2.09) but it is not clear if the estimate was adjusted for tobacco smoking, age, sex, and gender, although those characteristics seemed similarly distributed between cases and controls.¹⁹ Weaker associations between rs1800871 and lung cancer have also been reported in a Chinese population homozygous for the rare allele (aOR: 1.38, 95% CI: 0.57–3.38)²⁰ and among non-Hispanic Caucasians with at least 1 variant T allele (aOR: 1.43, 95% CI: 0.78–2.63).²¹ Despite differences in interpreting the association between *IL10* rs1800871 and lung cancer, point estimates from published studies (*i.e.*, OR \sim 1.4) are comparable with our own estimate (aOR: 1.5, 95% CI: 0.96–2.2), and the consistency between these estimates (p heterogeneity > 0.99) further suggests that *IL10* rs1800871 might be associated with elevated lung cancer risk, but the magnitude is not large.

IL-10 expression in oropharyngeal squamous cell carcinoma (SCC) has been inversely associated with survival²² and tumor grade and stage.²³ However, these studies do not rule out the possibility of association through altered cytokine expression as a result of somatic mutations within the tumor. Several studies of germline mutations suggest that *IL10* polymorphisms, which are associated with periodontitis,^{24,25} may also be associated with tongue cancer²⁶ and oral neoplasms.²⁷ *IL10* rs1800896, which is not in high linkage disequilibrium with rs1800871 ($r^2 = 0.30$), was associated with oral SCC in a hospital-based case-control study (aOR: 2.65, 95% CI: 1.28–5.46).²⁸ Another study from Sichuan, China reported that compared with the rs1800871 TT genotype, the CC genotype (which is more common in Asian populations) was associated with oral cancer (OR: 1.45, 95% CI: 0.88–2.39).²⁹ Recalculating the estimate with the CC genotype as the referent group yields a measure of association (OR: 0.69, 95% CI: 0.42–1.14) which is nearly identical to the effect estimated in our study (aOR: 0.69, 95% CI: 0.50–0.95).

Our results suggest that *IL1B* rs16944 may predict lung cancer, consistent with published reports,^{30–32} but our association did not hold after correction for multiple comparisons. Based on a large, multicenter case-control study, *IL1B* rs1143627 (linkage disequilibrium with rs16944: $r^2 = 0.94$) was not associated with lung cancer, suggesting that our find-

ing for rs16944 may be due to chance.³³ It is possible, though, that the association of lung cancer with *IL1B* polymorphisms could involve a pathway that is not primarily mediated by rs1143627.^{34,35}

The involvement of *TNF* polymorphisms in cancer has been reported for several malignancies, including lymphomas^{6,36,37} and lung cancer, though more null results have been reported for lung cancer^{18,21,32,38} than non-null associations.³⁹ The most consistent associations seem to come from gastric cancer studies. A meta-analysis of *TNF* polymorphisms reported positive associations for rs1800629 (summary OR [sOR]: 1.49, 95% CI: 1.11–1.99) and rs1799724 (sOR: 1.57, 95% CI: 0.91–2.70).⁵ We observed a similar association for rs1800629 in our gastric cancer sample (aOR: 1.3, 95% CI: 0.74–2.4). The apparent lack of published associations for rs1799964 could be because it may be most noticeable among nonsmokers, for whom the strong effect of tobacco-smoking is not as important a risk factor.

The difference in effects estimated between never smokers and ever smokers in our study suggests that smoking can modify the rate ratios between some inflammation-related SNPs and smoking related-cancers. In our pooled analysis, for example, smoking-related cancers were less common among never smokers with the *TNF* rs1799964 CC genotype, and the variant C allele did not appear protective among ever smokers (Table 2). Less than multiplicative smoking-SNP interactions were also suggested between lung cancer and *IL10* rs1800871, oropharyngeal cancer and *IFNG* rs2069705, and esophageal cancer and *IFNGRI* rs11914.

The mechanisms underlying these observations are unknown. Genetic polymorphisms in the numerous pathways involved in carcinogenesis affect cancer risk, and tobacco smoking may modify these effects.^{9,10,40,41} The epithelial cells from many of the organs in our study are repeatedly exposed to components of tobacco smoke or their metabolic byproducts, which are carcinogenic,⁴² known to cause vasoconstriction,⁴³ inhibit cell proliferation and angiogenesis,^{44,45} and are potent inducers of inflammation.^{46,47} The high levels of free radicals contained in⁴⁸ and generated by tobacco smoke can lead to cancer through oxidative DNA damage mediated by inflammation-associated production of reactive oxygen species.⁴⁹

Promoter polymorphisms of *TNF* are fairly numerous and in linkage disequilibrium with each other and with nearby genes,⁵⁰ which may have cooperative effects,⁵¹ potentially complicating the interpretation of single-SNP associations. One of the most commonly studied *TNF* SNPs is the rs1800629 G-308A polymorphism.⁵² The variant rs1799964 C allele, which is not in linkage disequilibrium with the G-308A polymorphism ($r^2 = 0.07$), appears to be associated with increased *TNF* expression.⁵³ *TNF* is an inflammatory cytokine and its expression by peripheral blood mononuclear cells has been demonstrated to increase following exposure to tobacco smoke.⁵⁴ Interestingly, *TNF* was expressed at higher levels in cells from nonsmokers than smokers at all time-points, which lends potential biologic support for our

observation that smoking-related cancers were less common among never smokers with the putative high expression *TNF* rs1799964 CC genotype. Haplotype studies of the gene encoding the anti-inflammatory cytokine IL-10 suggest that the GCC haplotype (*i.e.*, rs1800896 G, rs1800871 C, and rs1800872 C) is associated with high IL-10 production.^{55,56} Although haplotype data were unavailable in the LA study, the rs1800871 variant T allele associated with low production appeared to be an important SNP for lung cancer among never smokers, whereas the variant did not seem to increase risk among ever smokers.

The Van Dyke study of NSCLC among women¹⁸ reported associations for 6 SNPs that were also genotyped in our study (*IL1B* rs1143627 and rs16944; *TNF* rs1799964 and rs1800629; *LTA* rs909253; and *IL10* rs1800871). The estimated magnitude and direction of association for these SNPs were similar to our own estimates, both overall and among women, although comparability of our *TNF* results was affected by the small number of women with the variant C allele, resulting in unstable estimates. Although the majority of our SNPs do not result in amino acid substitutions, cancers of the oropharynx and lung appear to be associated with *IL10* rs1800871. The SNPs may influence cancer in part through modifying transcription and/or translation. Synonymous SNPs have been demonstrated to alter protein structure by affecting RNA splicing⁵⁷ and the stability^{58,59} and translation rate⁶⁰ of mRNA. However, further work assessing how these SNPs may affect transcription, translation, and protein conformation would help shed light on these hypothesized mechanisms.

Our study design and analytic strategy had a number of strengths and weaknesses. The associations in our study are subject to confounding and biases related to information ascertainment and subject selection. We included ethnicity as a covariate in regression models to address admixture but the effects of population stratification may still residually confound our estimates, particularly for SNPs with allele frequencies that differ greatly between ethnicities, such as *IL10* rs1800871 and *IFNG* rs2069705. The association we observed between lung cancer and *IL10* rs1800871, for example, might be partially due to uncontrolled differences in ethnicity. However, the ethnic diversity of the LA study facilitated examination of ethnicity-specific ratios of odds ratios, which were consistent in magnitude across ethnicity (except for African-Americans, for whom no never smoking lung cancer cases were observed with the risk genotype), suggesting that tobacco smoking and the recessive *IL10* rs1800871 genotype may interact on a less than multiplicative scale.

Because *TNF* rs1799964, which appeared to be an important SNP for nonsmokers in pooled analysis, does not appreciably vary across ethnic groups, it seems unlikely that population stratification was a significant problem. We attempted to minimize information bias by using strict inclusion and exclusion criteria for cases and controls, and by implementing stringent quality control measures in our laboratory. We

also used trained interviewers who used standardized questionnaires to collect detailed information on potential risk factors and related covariates to address confounding. Differential recollection of exposures for cases and controls could result in misclassification bias. Although we included pack-years of tobacco smoking in regression models to address residual confounding among ever smokers, our estimates may still be confounded by the strong effect of smoking. While we selected SNPs based on a pathway-based approach, additional markers per gene selected over a range of important regions (*e.g.*, splice sites, promoter areas, and tagging SNPs) would have been helpful to better characterize a gene, evaluate haplotypes, and ascertain whether an observed association could be mediated through linkage with SNPs.

The inverse association estimated for the *TNF* rs1799964 C variant among never smokers (Table 2) may reflect a true gene-environment interaction (in which the *TNF* rs1799964 CC genotype may afford some protection only in the absence of tobacco smoking) but a number of problems need to be considered. Data were sparse for some strata across ethnicity, even among the 920 cases and 825 controls who were self-identified as White. In particular, there were few observations of never smokers with the *TNF* rs1799964 CC variant genotype (especially true among cases), and none of the confidence intervals for these strata excluded the null when there were less than 5 observations per cell. The >60-fold ratios of the upper and lower 95% confidence limits illustrate the instability of some of these genotype-smoking estimates.

Cancer is a multifactorial process and a simple deterministic SNP-cancer association is unlikely. Given the number of comparisons that we made, our results could have been entirely due to chance. Therefore, we used 2 Bayesian approaches to account for Type I error, which suggest that our results are not purely chance findings. An inherent feature of Bayesian correction, though, is the use of subjective prior probabilities, which is susceptible to publication bias.⁶¹ Alternatively, our results may be due to other genetic and/or epigenetic mechanisms (*e.g.*, gene amplification, translocation, loss of heterozygosity, DNA methylation, genomic imprinting, and histone modification).^{62,63} These reasons underscore the importance of considering our results in the broader context of existing knowledge and studies and not to overemphasize the results of a particular study.

Differential participation of cases and controls and their willingness to donate samples for DNA analysis may have created selection bias. Of the 9 smoking-related sites in our study, esophageal, liver, lung, and stomach cancers have very low 5-year survival rates, reflected in the high percentage of cases in our study who died before they could be interviewed. Selection bias may exist if factors related to participation were differentially associated with exposure (*e.g.*, SNP genotype) for cases and controls. However, because a potential participant's genotype would have been unknown at time of recruitment, this type of bias seems unlikely. Selection bias could have occurred if a particular genotype was associated

with survival. However, it is unknown if the study was biased by this type of selection because a literature search did not yield sufficient information on the prognostic value of the SNPs. However, tumor grade and stage did not appear to vary by SNP genotype (Supporting Information Table S7). The relatively small number of cases and controls in the Taixing and MSKCC studies resulted in low statistical precision, and it had less than 80% power to detect main effect odds ratios of 1.5 or 0.67 for many of the SNP-cancer associations. Of the 4 SNPs in the Taixing study with sufficiently high minor allele frequencies to have at least 80% power (*IL10* rs1800871, *IL10* rs1800872, *LTA* rs909253, and *IL6* rs1800796), the 95% confidence intervals from the multivariate model included both positive and inverse associations. One SNP in the MSKCC study with sufficiently high minor allele frequency (*IL1B* rs1143627) was associated with bladder cancer (aOR = 4.3), but with an extremely wide confidence interval (95% CI: 1.3–14) (the association did not pass multiple comparisons correction) and was, therefore, not reported. The LA study had better precision due to its relatively large

sample size for assessing main and smoking-stratified effects for the lung and UADT sites. The ethnic diversity in the pooled sample and the LA study also allowed us to examine ethnicity-specific SNP-cancer associations to assess admixture. Additional strengths include the ability to examine SNP-cancer associations across a number of different cancer sites; the use of a population-based study design for the LA and Taixing studies; and control for multiple comparisons using 2 Bayesian approaches.

Our results if valid suggest that *TNF* rs1799964 is inversely associated with smoking-related cancers among never smokers, and that *IL10* rs1800871 is a susceptibility marker for lung cancer among never smokers, and for oropharyngeal cancer among ever smokers.

Acknowledgements

We would like to thank Dr. Thomas M. Mack and Dr. Wendy Cozen for their contributions for the LA population-based lung and UADT case-control study. We are indebted to the studies' participants for their time and dedication.

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