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Interaction of cigarette smoking and carcinogen-metabolizing polymorphisms in the risk of colorectal polyps

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The causal role of cigarette smoking in the risk of colorectal neoplasm has been suggested but not established. In a case–control study including 2060 colorectal polyp patients and 3336 polyp-free controls, we evaluated 21 functional genetic variants to construct a tobacco-carcinogen-metabolizing genetic risk score. Data regarding cigarette smoking were obtained through telephone interviews. Cigarette smoking was associated with an elevated risk of both adenomas and hyperplastic polyps. The association with smoking was stronger in participants with a high carcinogen-metabolizing risk score than those with a low risk score. Smoking 30 or more cigarettes per day was associated with a 1.7-fold elevated risk of any polyps (95% confidence interval = 1.3–2.2) among those with a low genetic risk score and 2.9-fold elevated risk (95% confidence interval = 1.8–4.8) among those with a high genetic risk score ($P_{interaction}$ = 0.025). A similar pattern of interaction was observed in analyses conducted separately for those with adenomas only ($P_{interaction}$ = 0.039) and hyperplastic polyps only ($P_{interaction}$ = 0.024). Interaction between carcinogen-metabolizing genetic risk and cigarette smoking was found in relation to high-risk adenomas ($P_{interaction}$ = 0.010) but not low-risk adenomas ($P_{interaction}$ = 0.791). No apparent interaction was found for duration of smoking. This study shows that the association between cigarette smoking and colorectal polyp risk is modified by tobacco-carcinogen-metabolizing polymorphisms, providing support for a causal role of cigarette smoking in the etiology of colorectal tumors.

Introduction

Cigarette smoking has been linked to the risk of colorectal cancer and adenomas (1,2). Colorectal adenomas are precursors of colorectal cancer (3,4); they can develop into colorectal cancer mostly through an adenoma–carcinoma sequence (5). Recent evidence suggests that some hyperplastic polyps (HPP) may develop into cancer via serrated or microsatellite instable pathways (6,7). It is common for an individual to have both synchronous HPP and adenomas (8,9). It has been shown that patients with both synchronous HPP and adenomas are more likely to have recurrence of adenomas than those with either adenomas or HPP (9). Studies have shown cigarette smoking to be more strongly associated with the risk of both synchronous HPP and adenomas and the risk of HPP only than adenomas only. However, the association between genetic variation in genes encoding tobacco-carcinogen-metabolizing enzyme and the risk of colorectal adenoma and HPPs has been inconsistent.

Cigarette smoke contains a variety of carcinogenic compounds, including polycyclic aromatic hydrocarbons, heterocyclic amines, aromatic amines and nitrosamines (10). Most tobacco carcinogens require metabolic activation by Phase I enzymes before they are able to react with DNA. This metabolic activation often is initiated by cytochrome P450 enzymes (CYPs, i.e. CYP1A2 and CYP1B1). These active metabolites are then detoxified by Phase II enzymes, including UDP-glucuronosyltransferases (UGT) and glutathione-S-transferases (GST), and then excreted from the body (10,11). Aryl hydrocarbon receptor (AhR) is an important xenobiotic signaling mediator to enhance the expression of both Phase I and Phase II enzymes (12), which affects tobacco-carcinogen metabolism. Internal doses of tobacco-carcinogen exposure, and the resulting biological effects, are influenced by the balance of enzymes that activate and detoxify tobacco carcinogens. The amount and function of key carcinogen-metabolizing enzymes are determined, in part, by genetic polymorphisms of genes encoding these enzymes. Categorization of tobacco-carcinogen-metabolizing enzyme genotypes provides a tool to classify participants into groups with different internally active carcinogen-exposure levels despite the same external exposure level. In other words, if cigarette smoking is causally associated with colorectal polyp risk, we would expect that this association may be modified by genetic polymorphisms of enzymes involved in the metabolism of tobacco carcinogen. Individuals with a high internal exposure to biologically active tobacco carcinogen, as predicted by both external exposure assessment and tobacco-carcinogen-metabolizing pattern, may be at an elevated risk of polyps.

We used data and DNA samples collected in the Tennessee Colorectal Polyp Study (TCPS) to test this hypothesis. Because tobacco-carcinogen-metabolizing genotypes are established through random assortment during gamete formation, they should be independent of external exposure to tobacco carcinogens and are unlikely to be related to confounding factors. Our study is consistent with Mendelian randomization analysis in studying gene–environment interactions in the risk of diseases, which helps reduce potential biases associated with observational studies.

Abbreviations: CI, confidence intervals; CYP, cytochrome P450 enzymes; EPHX, microsomal epoxide hydrolases; GST, glutathione-S-transferases; HPP, hyperplastic polyp; NAT, *N*-acetyltransferases; OR, odds ratio; SNP, single nucleotide polymorphisms; TCPS, Tennessee Colorectal Polyp Study; UGT, UDP-glucuronosyltransferases.

Participants and methods

Recruitment of study participants

The TCPS is a colonoscopy-based case–control study conducted in Nashville, TN. Detailed methods used in this study have been described elsewhere (13,14). Eligible participants of age 40–75 years were identified from patients scheduled for colonoscopy at an academic medical center (Vanderbilt University Medical Center) and a Veterans Affairs medical center (Tennessee Valley Healthcare System, Nashville, TN) between 1 February 2003 and 26 March 2010. The study was approved by the Institutional Review Boards of all participating institutions.

Excluded from our study were participants with genetic colorectal cancer syndromes or with prior history of inflammatory bowel disease, colorectal adenomas, or any cancer other than non-melanoma skin cancer. Among 10 074 eligible participants, 7330 (72.8%) provided written informed consent, of whom 6331 (86.4% of responders) completed a telephone interview. Based on the colonoscopy and pathologic findings, polyp cases were categorized as follows: (i) cases with HPP only, (ii) cases with adenoma only and (iii) cases with both synchronous adenoma and HPP. Eligible controls were participants who had received a complete colonoscopy reaching the cecum and were found to be polyp free. Twenty-four cases were excluded due to missing data. Among

those with completed interviews, 5396 participants were genotyped. The current analyses include 1160 cases with adenoma only, 532 cases with HPP only, 368 cases with both synchronous adenoma and HPP and 3336 polyp-free controls. Based on endoscopic report, advanced adenomas were defined as adenomas with a diameter ≥ 1 cm, high-grade dysplasia, or tubulovillous or villous morphology.

Assessment of cigarette smoking and other lifestyle factors

After colonoscopy, trained interviewers conducted a standardized telephone interview to obtain information regarding medication use, demographics, medical history and selected lifestyle factors, including detailed information about cigarette smoking (13). Briefly, regular cigarette smoking (or ever smoking) was defined as smoking at least one cigarette per day for at least 3 months continuously. Former smokers were regular smokers who had stopped for at least 1 year before colonoscopy. Information was collected about age at initiation of cigarette smoking, whether the participant was still smoking regularly, age at which regular smoking stopped, average number of cigarettes smoked per day (either currently or before quitting) and maximum number of cigarettes smoked per day regularly during the participant's lifetime. Regular alcohol drinking was defined as consuming five or more drinks per week for 12 months continuously. Regular non-steroidal anti-inflammatory drug (NSAID) users were defined as those using NSAIDs at least three times each week for at least 12 months continuously. All cut-points for exposure variables were based on distributions in control participants.

Genetic variant selection and genotyping assays

In this study, we selected 12 key enzymes involved in tobacco-carcinogen metabolism (Table I). We attempted to identify all well-established functional genetic variants involved in the entire tobacco-carcinogen-metabolism pathway based on literature showing functional changes of enzymes by *in vitro* studies of these variants. Tobacco-carcinogen-metabolizing enzymes were categorized into Phase I and Phase II enzymes (Table I). We identified 27 functional variants of these enzymes, of which 25 were successfully genotyped. Those which failed genotyping were C1095A (3′UTR, rs15561) and T1088A (3′UTR, rs1057126) in *NAT1* gene. Four *NAT1* single nucleotide polymorphisms (SNPs), that is C97T (R33Stop), C190T (R64W), C620T (T207I) and A752T (D251V), were monomorphic in our population and thus were excluded. As a result, two *NAT1* SNPs remained in the analysis, that is *NAT1*14* (G560A, rs4986782) and *NAT1*15* (C559T, rs5030839). The other 19 genetic variants included in our analysis were *AhR* (G1661A, rs2066853), *CYP1A2*1K* (C163A, rs762551), *CYP1B1*3* (G4329C, rs1056836), *CYP1B1*4* (A4393G, rs1800440), *CYP2C9*2* (C3608T, rs28371674), *CYP2C9*3* (A1075C, rs1057910), *CYP2E1* (C1055T, rs2031920), *SULT1A1*2* (G638A, rs9282861), microsomal epoxide hydrolase (*EPHX1*, T337C, rs1051740), *UGT1A7*9* (G343A, rs61261057), *GSTM1* homozygous deletion, *GSTT1* homozygous deletion, and 7 *NAT2* SNPs: (C282T, rs1041983), (A803G, rs1208), (C481T, rs1799929), (G590A, rs1799930), (G857A, rs1799931), (G191A, rs1801279) and (T341C, rs1801280).

We utilized genomic DNA extracted from blood or buccal cells for genotyping assays (15). All allelic gene polymorphisms were assessed by TaqMan OpenArray system. TaqMan OpenArray Assay-on-Demand reagents were available from Applied Biosystems (ABI) for all SNPs except *NAT2* G191A (rs1801279). Primers for *NAT2* G191A (rs1801279) polymorphisms are self-designed and synthesized by ABI (primers were GGAGTTGGGCTTAGAGGCTATTTT and CAGAAGTTGATTGACCTGGAGACA; probes were VIC-CCACCCCGGTTTC and FAM-CCCACCCTGGTTTC). Primers and probes for these SNPs were pre-loaded by ABI. DNA samples (2.5 µl) and TaqMan Universal PCR Master Mix (2.5 µl) were mixed in a 384-well plate. PCR was performed, consisting of an initial denaturation step at 93°C for 10 min and 50 cycles of 95°C for 45 s, 94°C for 13 s and 53°C for 134 s, and post-PCR hold at 25°C for 2 min. The fluorescence imaging of genotyping plates could be performed together with the ABI OpenArray™ NT Imager. Allele frequencies were determined by ABI OpenArray software (AutoCaller™). Laboratory staffs were blinded to the case–control status of samples. Quality-control protocols for genotyping assays were employed as described previously (15). Briefly, each 384-well plate contained 4 water blanks, 8 CEPH 1347-02 DNA and 16 blinded quality-control samples. Blinded quality-control samples were taken from the second tube of samples included in the study. Quality-control samples were distributed across the 384-well plates. Concordance rate for blinded quality-control samples was 100% for all SNPs. In addition, DNA of 45 Caucasian samples included in the HapMap and Perlegen projects was purchased from Coriell Cell Repositories (http://locus.umdnj.edu/ccr/) and genotyped for all 21 SNPs. Average consistency rates of the 21 SNPs were 99.3% compared with data from HapMap (http://www.hapmap.org) and Perlegen (http://genome.perlegen.com). Call rates for all SNPs were >95% (mean call rate = 97.5 %) and all genotypes were consistent with Hardy–Weinberg equilibrium with $P > 0.05$ in controls.

Table I. Polymorphisms in selected polycyclic aromatic amine metabolizing genes and their effect on enzyme function

	Variant (alleles)[a,b]	Risk allele frequency[c]	Amino acid change	Effect of risk allele on metabolizing enzymatic activity
Phase I enzymes				
AHR	rs2066853(G/**A**)	0.183	R554K	Higher inducibility (12)
CYP1A2	rs762551 (C/**A**)	0.716	NA	Increasing enzymatic activity (45)
CYP1B1	rs1056836 (**G**/C)	0.532	V432L	Increasing mRNA expression (46)
	rs1800440 (**A**/G)	0.827	N453S	Increasing enzymatic activity (47)
CYP2C9	rs28371674 (**C**/T)	0.118	R144C	Increasing enzymatic activity (48)[d]
	rs1057910 (**A**/C)	0.059	I359L	Increasing enzymatic activity (49)[d]
CYP2E1	rs2031920 (C/**T**)	0.022	NA	Increasing enzymatic activity by enhancing the transcription (50,51)
Phase II enzymes				
NAT1[e]	rs4986782 (G/**A**)	0.017	R187Q	Slow acetylation; Decreasing enzymatic activity (52)
	rs5030839 (C/**T**)	0.003	R187X	Slow acetylation; Decreasing enzymatic activity (52)
NAT2[e]	rs1041983 (C/**T**)	0.335	Y94Y	The combined effect of all 7 SNPs were categorized to slow acetylation(reference), intermediate acetylation and rapid acetylation (Increasing enzymatic activity) (52,53)
	rs1208 (G/**A**)	0.426	R268K	
	rs1799929 (C/**T**)	0.409	L161L	
	rs1799930 (G/**A**)	0.711	R197Q	
	rs1799931 (G/**A**)	0.048	G286E	
	rs1801279 (G/**A**)	0.008	R64Q	
	rs1801280 (T/**C**)	0.570	I114T	
EPHX1	rs1051740 (T/**C**)	0.290	T213H	Decreasing enzymatic activity (54)
SULT1A1	rs9282861 (G/**A**)	0.236	R213H	Decreasing enzymatic activity (55,56)
UGT1A7	rs61261057 (G/**A**)	0.002	G115S	Decreasing enzymatic activity (57)
GSTM1	Homozygous deletion	0.089	Null	Depleted enzymatic activity (58,59)
GSTT1	Homozygous deletion	0.145	Null	Depleted enzymatic activity (59)

[a]Database of SNPs (http://www.ncbi.nlm.nih.gov/SNP/).
[b]Risk allele is highlighted in bold.
[c]Frequency of the minor allele among controls in the TCPS.
[d]Database of SNPs (http://www.cypalleles.ki.se/cyp2c9.htm).
[e]Database of SNPs (http://louisville.edu/medschool/pharmacology/nat/). Details for phenotype imputation shown in Supplementary Appendices 3 and 4, available at *Carcinogenesis* Online.

Statistical analysis

Each polymorphism was tested in controls to ensure fitting with Hardy–Weinberg equilibrium. We assigned a risk allele to each genetic variant based on the effect on tobacco-carcinogen metabolism. A risk allele was defined as an allele that either increases the activity/amount of a Phase I enzyme or reduces the activity/amount of a Phase II enzyme. For genetic variants other than in *NAT1* and *NAT2*, each participant received a score of 0, 1 or 2 for carrying zero, one or two risk alleles, respectively (Supplementary Appendix 1, available at *Carcinogenesis* Online). *NAT1* and *NAT2* phenotype statuses were derived based on the combination of several SNPs provided in Supplementary Appendices 3 and 4, available at *Carcinogenesis* Online. Predicted *NAT1* phenotypes were classified 'slow' with a genetic risk score of '0' or 'non-slow' with a score of '1'. Derived *NAT2* phenotypes were classified 'slow', 'intermediate' and 'fast' with a score of '0', '1' and '2', respectively. The tobacco-carcinogen-metabolizing genetic score was derived by summing individual risk scores across all Phase I activation enzymes (*AhR, CYP1A2, CYP1B1, CYP2C9* and *CYP2E1*) and Phase II enzymes (*NAT1, NAT2, EPHX1, SULT1A1, UGT1A7, GSTM1* and *GSTT1*; total possible score = 19). We used tertile cut-points for overall carcinogen-metabolizing scores of '9' and '12' to classify participants into low-risk genetic risk group (score ≤ 9), intermediate genetic risk group (score 10–12) and high-risk genetic risk group (score > 12; Supplementary Appendix 2, available at *Carcinogenesis* Online).

General linear models and Mantel-Haenszel chi-square tests were used to compare distribution of demographic characteristics and known colorectal-cancer risk factors across carcinogen-metabolizing risk groups, as well as between case and control groups, with adjustment for age and sex when appropriate. Unconditional logistic regression models were used to estimate odds ratios (OR) and their 95% confidence intervals (CI) for the association between genetic variants and smoking with polyp risk. ORs were adjusted for selected confounders for colorectal adenoma that showed significantly different distributions between cases and controls. Variables selected for multivariate analysis included age (40–49, 50–59, 60–64, ≥65), sex, study site (academic medical center, VA medical center), educational attainment (high school or less, some college, college graduate, graduate or professional education), red meat intake (g/day, continuous), regular alcohol consumption (never/former/current), body mass index (BMI, continuous), regular exercise (yes/no), regular NSAID use (never/former/current), year of recruitment and recruitment before or after colonoscopy. *P* values for linear trend tests were derived by treating categorical variables as continuous parameters in the models (16). Likelihood ratio tests of multiplicative interaction for categorical variables were used to compare models with and without interaction terms (16). *P* values of ≤0.05 (two-sided probability) were considered statistically significant. All analyses were conducted using SAS statistical software (version 9.2; SAS Institute, Cary, NC).

Results

Table I summarizes Phase I and Phase II tobacco-carcinogen-metabolizing enzymes and functional genetic variants of these enzymes included in this analysis. Highlighted in bold are risk alleles which may increase internal carcinogen exposure, based on results of *in vitro* functional studies.

Distributions of selected demographic characteristics and major risk factors for colorectal cancer are presented in Table II for the three polyp case groups and the polyp-free controls. More controls than cases were recruited from Vanderbilt Medical Center than the VA medical center. Compared with controls, polyp cases were more likely to be male, smokers and regular alcohol consumers and less probable to use NSAIDs regularly. Cases also had higher BMI and lower educational attainment than controls. Cases with any adenoma were older than controls, whereas cases with HPP only were similar in age to controls. Case–control distributions of race and indication for colonoscopy were comparable.

No apparent association was observed between the tobacco-carcinogen-metabolizing risk score and the risk of any-polyp groups evaluated in the study including adenoma only, HPP only and those with both synchronous adenoma and HPP (Table III). Associations between risk of polyps and the Phase I or Phase II carcinogen-metabolizing risk score were also null (data not shown).

All cigarette-smoking variables were associated with risk of all polyps combined regardless of the category of carcinogen-metabolizing risk score (Table IV). Association with cigarette smoking was stronger among participants with a high carcinogen-metabolizing risk than those with a low score. Tests for multiplicative interactions were statistically significant for pack-years of cigarette smoking ($P_{interaction}$ = 0.032) and cigarettes smoked per day ($P_{interaction}$ = 0.025). No apparent interaction between genetic risk score and years of smoking was observed and thus it is probable that the significant interaction with pack-years smoked may be explained primarily by intensity

Table II. Selected demographic characteristics and major known risk factors for colorectal cancer by study groups, TCPS, 2003–2010

Characteristic	Controls	Polyp cases			
	(N = 3336)	Adenoma only (*N* = 1160)	HPP only (*N* = 532)	Both (*N* = 368)	*P* value[a]
Study site (%)					
Vanderbilt University	73.8	61.3	54.0	47.3	<0.001
Veterans Affairs	26.2	38.7	46.0	52.7	
Age [years, mean (SD)]	57.2 (7.6)	59.2 (7.4)	57.1 (6.9)	58.9 (6.5)	<0.001
Sex (female, %)	43.8	27.2	30.1	18.8	<0.001
Indications for colonoscopy (%)[b]					
Screening	58.6	57.7	56.1	52.4	0.062
Other	41.4	42.3	43.9	47.6	
Educational attainment (%)[b]					
High school or less	24.5	29.7	33.1	37.7	<0.001
Some college	28.7	27.0	31.4	38.5	
College graduate	20.4	21.9	18.5	12.7	
Graduate or professional education	26.5	21.4	17.0	11.0	
Race (white, %)	89.6	88.7	91.9	91.9	0.114
Colorectal cancer family history (%)[b]	8.4	9.9	8.1	10.4	0.378
Regular cigarette smoking (%)[b]	49.1	57.0	72.6	75.7	<0.001
Regular alcohol consumption (%)[b]	43.1	47.0	50.7	45.2	0.009
Body mass index (kg/m^2, mean)	28.1	28.7	28.8	29.1	<0.001
Regularly exercised (%)[b]	57.5	52.8	53.3	48.0	<0.001
Regular NSAID use (%)[b]	51.6	52.8	53.3	48.0	0.025
Red meat intake (g/day, mean)	57.4	73.4	79.8	85.3	<0.001

Both, synchronous adenoma and HPPs.
[a]Derived from analysis of variance for continuous variables and chi-square test for categorical variables.
[b]Standardized by age (40–49, 50–59, 60–64 and ≥65 years) and sex distribution of all study participants.

but not duration of cigarette smoking. Because of this, only results for smoking status and number of cigarettes smoked per day are presented in subsequent tables.

Associations with cigarette smoking by carcinogen-metabolizing risk categories were analyzed separately for three case groups: (i) adenoma only, (ii) HPP only and (iii) both synchronous adenoma and HPP (Table V). Multiplicative interactions at $P \leq 0.05$ were found for number of cigarettes smoked per day in the adenoma-only and HPP-only groups. Although associations with smoking were stronger for those with both synchronous adenoma and HPP than with the adenoma-only or HPP-only groups, no significant interaction was observed for both synchronous adenoma and HPP. Similar to the results for all polyps combined, no apparent interaction was observed for years of smoking in these subgroup analyses (data not shown).

Strong association and significant interaction of carcinogen-metabolizing genetic risk score and smoking were found for clinically important adenomas, that is advanced or multiple adenomas, but not for non-advanced and single adenomas (Table VI). Most of the joint effects for non-advanced and single adenomas were not significant, although the sample size ($N = 578$) was slightly larger than advanced or multiple adenomas group ($N = 531$).

Discussion

In this study, we showed that genetically controlled tobacco-carcinogen-metabolizing pattern modifies the association between cigarette smoking and the risk of colorectal polyps, including adenomas and HPP. Among patients with adenomas only, this interaction was found for high-risk adenomas, that is advanced or multiple adenomas, but not for low-risk adenomas, that is non-advanced and single adenomas. Our results provide strong evidence to implicate an etiological role of tobacco-carcinogen exposure in the formation and in the progression of colorectal neoplasia.

Table III. Association between carcinogen-metabolizing scores and colorectal polyp risk, the TCPS[a]

Carcinogen-metabolizing risk score by tertile	Control ($N = 3,336$)	Polyp cases							
		All polyps ($N = 2060$)		Adenoma only ($N = 1160$)		HPP only ($N = 532$)		Both ($N = 368$)	
	n	*n*	OR (95%CI)[a]	*n*	OR (95%CI)[a]	*n*	OR (95%CI)[a]	n	OR (95%CI)[l]
T1 (0–9)	1218	756	1.0 (reference)	427	1.0 (reference)	186	1.0 (reference)	143	1.0 (reference)
T2(10–12)	1775	1075	1.0 (0.9–1.1)	611	1.0 (0.8–1.1)	288	1.1 (0.9–1.3)	180	0.8 (0.7–1.1)
T3(13–19)	343	225	1.1 (0.9–1.3)	122	1.1 (0.8–1.3)	58	1.2 (0.9–1.6)	45	1.3 (0.9–1.9)
Trend test			$P = 0.654$		$P = 0.933$		$P = 0.287$		$P = 0.096$

Both, synchronous adenoma and HPP.
[a]Adjusted for age, sex, study sites, educational attainment, alcohol consumption, BMI, physical activity, regular NSAID use, red meat intake, total energy intake, year of recruitment and recruitment before or after colonoscopy.

Table IV. Risk of all polyps combined in association with cigarette smoking by carcinogen-metabolizing risk category, the TCPS, 2003–2010

Smoking	Carcinogen-metabolizing risk score					
	Low risk		Intermediate risk		High risk	
	n[a]	OR (95% CI)[b]	*n*[a]	OR (95% CI)[b]	*n*[a]	OR (95% CI)[b]
Smoking status						
Never	261/656	1.0 (reference)	344/916	0.9 (0.8–1.1)	67/182	0.9 (0.7–1.3)
Former	286/405	1.4 (1.1–1.7)	406/630	1.3 (1.1–1.6)	88/117	1.6 (1.2–2.2)
Current	207/156	2.9 (2.2–3.8)	328/227	3.0 (2.4–3.9)	69/43	3.4 (2.2–5.2)
			Test of interaction, $P = 0.341$			
Pack-years						
Never	261/656	1.0 (reference)	344/916	0.9 (0.8–1.1)	67/182	0.9 (0.7–1.3)
1–9	101/156	1.6 (1.2–2.1)	137/277	1.2 (0.9–1.6)	36/54	1.6 (1.0–2.5)
10–29	149/178	1.8 (1.4–2.4)	213/268	1.7 (1.3–2.1)	34/65	1.2 (0.7–1.8)
≥30	242/227	2.0 (1.5–2.5)	380/307	2.3 (1.8–2.9)	84/40	4.0 (2.6–6.1)
			Test of interaction, $P = 0.032$			
Cigarettes per day						
Never	261/656	1.0 (reference)	344/916	0.9 (0.8–1.1)	67/182	1.0 (0.7–1.3)
1–19	183/211	2.1 (1.6–2.7)	269/345	1.8 (1.4–2.2)	44/75	1.4 (0.9–2.1)
20–29	151/187	1.5 (1.1–1.9)	233/292	1.5 (1.2–1.9)	64/54	2.3 (1.6–3.5)
≥30	159/163	1.7 (1.3–2.2)	230/215	1.8 (1.4–2.3)	49/30	2.9 (1.8–4.8)
			Test of interaction, $P = 0.025$			
Years of cigarette smoking						
Never	261/656	1.0 (reference)	344/916	0.9 (0.8–1.1)	67/182	1.0 (0.7–1.3)
1–14	86/159	1.2 (0.9–1.6)	110/284	0.9 (0.7–1.2)	35/54	1.6 (1.0–2.6)
15–24	83/122	1.4 (1.0–2.0)	104/158	1.3 (1.0–1.8)	21/32	1.5 (0.8–2.6)
25–34	111/121	2.0 (1.5–2.7)	181/185	2.1 (1.6–2.8)	35/35	2.1 (1.3–3.6)
≥35	212/159	2.4 (1.8–3.3)	337/229	2.9 (2.3–3.7)	63/39	3.2 (2.2–4.7)
			Test of interaction, $P = 0.294$			

[a]n, number of cases/controls. The total number of subjects was 5380 (including 2054 cases and 3326 controls). Sixteen subjects were deleted because of missing data.
[b]Adjusted for age, sex, study sites, educational attainment, alcohol consumption, BMI, physical activity, regular NSAID use, red meat intake, total energy intake, year of recruitment and recruitment before or after colonoscopy.

Table V. Joint association of cigarette smoking with risk of polyps by subtypes, the TCPS, 2003–2010

Cigarette smoking	Carcinogen-metabolizing risk category					
	Low risk		Intermediate risk		High risk	
	n^a	OR (95% CI)[b]	n^a	OR (95% CI)[b]	n^a	OR (95% CI)[b]
Adenoma only (N = 1161)						
Smoking status						
Never	183/656	1.0 (reference)	237/916	0.9 (0.7–1.1)	43/182	0.9 (0.6–1.3)
Former	162/405	1.1 (0.8–1.4)	230/630	1.0 (0.8–1.3)	48/117	1.2 (0.8–1.8)
Current	81/156	1.6 (1.1–2.3)	144/227	1.9 (1.4–2.6)	30/43	2.1 (1.2–3.5)
			Test of interaction, P = 0.141			
Cigarettes per day						
Never	183/656	1.0 (reference)	237/916	0.9 (0.7–1.1)	43/182	0.9 (0.6–1.3)
1–19	92/211	1.4 (1.0–1.9)	140/345	1.3 (1.0–1.7)	25/75	1.1 (0.6–1.8)
20–29	74/187	1.1 (0.8–1.5)	110/292	1.0 (0.8–1.4)	27/54	1.5 (0.9–2.4)
≥30	77/163	1.1 (0.8–1.6)	123/215	1.4 (1.0–1.9)	26/30	2.0 (1.1–3.5)
			Test of interaction, P = 0.039			
HPP only (N = 532)						
Smoking status						
Never	50/656	1.0 (reference)	74/916	1.1 (0.7–1.6)	13/182	1.0 (0.5–1.8)
Ever	71/405	2.0 (1.3–2.9)	110/630	2.0 (1.4–2.9)	23/117	2.4 (1.4–4.2)
Current	64/156	5.1 (3.3–7.8)	103/227	5.4 (3.6–8.0)	22/43	6.1 (3.3–11.2)
			Test of interaction, P = 0.753			
Cigarettes per day						
Never	50/656	1.0 (reference)	74/916	1.1 (0.7–1.6)	13/182	1.0 (0.5–1.8)
1–19	63/211	3.9 (2.6–5.9)	83/345	3.0 (2.1–4.4)	12/75	1.9 (0.9–3.8)
20–29	36/187	1.9 (1.2–3.0)	73/292	2.7 (1.8–4.0)	17/54	3.3 (1.8–6.3)
≥30	36/163	2.1 (1.3–3.4)	56/215	2.4 (1.5–3.7)	16/30	6.1 (3.1–12.2)
			Test of interaction, P = 0.024			
Both (N = 368)						
Smoking status						
Never	28/656	1.0 (reference)	33/916	0.8 (0.5–1.4)	11/182	1.5 (0.7–3.2)
Ever	53/405	2.1 (1.3–3.5)	66/630	1.7 (1.1–2.8)	17/117	2.9 (1.5–5.6)
Current	62/156	7.7 (4.6–12.9)	81/227	6.2 (3.8–10.1)	17/43	7.4 (3.6–15.1)
			Test of interaction, P = 0.410			
Cigarettes per day						
Never	28/656	1.0 (reference)	33/916	0.8 (0.5–1.4)	11/182	1.5 (0.7–3.2)
1–19	28/211	2.9 (1.6–5.0)	46/345	2.7 (1.6–4.5)	7/75	2.3 (0.9–5.5)
20–29	41/187	3.5 (2.0–5.9)	50/292	2.9 (1.7–4.7)	20/54	6.0 (3.1–11.7)
≥30	46/163	3.8 (2.2–6.4)	51/215	3.1 (1.8–5.2)	7/30	3.9 (1.5–9.9)
			Test of interaction, P = 0.837			

Both, synchronous adenoma and HPP.
[a]n, number of cases/controls. Count may not sum up to total because of missing data.
[b]Adjusted for age, sex, study sites, educational attainment, alcohol consumption, BMI, physical activity, regular NSAID use, red meat intake, total energy intake, year of recruitment, and recruitment before or after colonoscopy.

We did not find any association of polyp risk with any individual genetic variant or with combined risk scores of these variants. Results from previous studies regarding the association of genetic factors with colorectal cancer risk (17–23) and adenoma risk (24–36) were largely null. Results for potential modifying effects of genetic factors on the association of cigarette smoking have been inconsistent. These inconsistent findings are not surprising because each of the genetic variants in the metabolizing pathway plays a small role in the activation or detoxification of tobacco carcinogens. Therefore, it is important to combine information from multiple genetic variants to capture the carcinogen-metabolizing pattern of each individual. In addition, because multiple comparisons have been made and many previous studies are small in sample size, the inconsistent results are hard to interpret. Therefore, in this study, we did not attempt to address interactions between individual genetic variants and cigarette smoking. No association between polyp risk and the genetic score was observed. However, a significant effect modification of the genetic score on the association between cigarette smoking and risk of colorectal polyps was found. We found that the effect modification of genetic factors on cigarette smoking may be due more to smoking intensity than smoking duration. Although this observation is not unexpected because internal exposure dose is more probable to be affected by intensity than duration of exposure, future research is needed to confirm this finding.

Our study, in general, fulfilled the three criteria required to apply Mendelian randomization analyses (39–42). First, the genotypic profile, as summarized by the carcinogen-metabolizing risk score, was independent of factors that may confound the association between cigarette-smoking intake levels or carcinogen-exposure levels and risk of colorectal polyps (39–41). We evaluated this criterion by analyzing the distribution of demographic characteristics and risk factors presented in Table I by carcinogen-metabolizing risk categories (Supplementary Appendix 2, available at *Carcinogenesis* Online). Our results show no difference between the low-, intermediate- and high-carcinogen-metabolizing risk categories in the distributions of all demographic characteristics and risk factors. These findings support successful Mendelian randomization of study participants. Regarding the second criterion, the carcinogen genetic risk profile must be related to risk of disease through the exposure of interest only and not through other pathways. Because many metabolizing genes are pleiotropic, they may be involved in the metabolism of other carcinogens. We selected functional SNPs involved in the metabolism of multiple tobacco carcinogens, including aromatic amines, polycyclic aromatic hydrocarbons and nitrosamines. In an attempt to measure the overall genetic variation in the metabolism of multiple tobacco carcinogens, we combined these SNPs to construct a genetic score. We believe this approach is appropriate because the

Table VI. Joint association of cigarette smoking and carcinogen-metabolizing risk categories in relation to adenoma risk, the TCPS, 2003–2010

Cigarette smoking	Total metabolizing risk category					
	Low risk		Intermediate risk		High risk	
	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]	n[a]	OR (95% CI)[b]
Advanced or multiple adenomas (N = 531)						
Smoking status						
Never	117/656	1.0 (reference)	131/916	1.1 (0.8–1.5)	27/182	0.9 (0.5–1.6)
Ever	89/405	1.3 (0.9–1.9)	117/630	1.3 (0.9–1.9)	27/117	1.4 (0.8–2.4)
Current	46/155	2.0 (1.2–3.2)	63/227	3.1 (2.1–4.6)	11/43	3.7 (2.0–7.0)
			Test of interaction, P = 0.010			
Cigarettes per day						
Never	117/656	1.0 (reference)	131/916	1.1 (0.8–1.5)	27/182	0.9 (0.5–1.6)
1–19	56/210	1.6 (1.0–2.4)	73/345	1.8 (1.2–2.6)	12/75	1.5 (0.7–2.9)
20–29	41/187	1.3 (0.8–2.1)	56/292	1.3 (0.9–2.0)	11/54	2.4 (1.3–4.5)
≥30	38/163	1.5 (0.9–2.4)	51/215	2.2 (1.4–3.2)	15/30	2.3 (1.1–4.9)
			Test of interaction, P = 0.132			
Single non-advanced adenomas (N = 578)						
Smoking status						
Never	111/656	1.0 (reference)	123/916	0.8 (0.6–1.1)	26/182	0.9 (0.6–1.5)
Ever	84/405	1.0 (0.7–1.4)	108/630	0.8 (0.6–1.1)	23/117	1.0 (0.6–1.6)
Current	40/156	1.3 (0.9–2.1)	52/227	1.1 (0.7–1.6)	10/43	1.1 (0.5–2.4)
			Test of interaction, P = 0.791			
Cigarettes per day						
Never	111/656	1.0 (reference)	123/916	0.8 (0.6–1.1)	26/182	0.9 (0.6–1.5)
1–19	52/211	1.4 (0.9–2.0)	65/345	1.0 (0.7–1.4)	11/75	0.8 (0.4–1.6)
20–29	39/187	0.9 (0.6–1.4)	52/292	0.8 (0.6–1.2)	11/54	1.0 (0.5–2.0)
≥30	33/163	0.9 (0.6–1.4)	43/215	0.8 (0.6–1.3)	11/30	1.5 (0.7–3.1)
			Test of interaction, P = 0.159			

[a]n, number of cases/controls. The total number of subjects was 4435 (including 1109 cases with adenoma only and 3326 controls). Count may not sum up to total because of missing data.
[b]Adjusted for age, sex, study sites, educational attainment, alcohol consumption, BMI, physical activity, regular NSAID use, red meat intake, total energy intake, year of recruitment, and recruitment before or after colonoscopy.

objective of this study was to evaluate interaction with tobacco smoking, not interaction with any single tobacco carcinogen. Furthermore, because multiple tobacco carcinogens have been linked to the risk of colorectal polyps, it would be more powerful to evaluate the overall association of polyp risk with tobacco smoke than the specific association of polyp risk with any single tobacco carcinogen. On the other hand, because these SNPs were selected from the pathway of tobacco-carcinogen metabolism, it is highly unlikely that the genetic score derived from the entire set of tobacco-carcinogen-metabolizing SNPs would be closely related to the metabolism of substrates other than tobacco carcinogens. In other words, the carcinogen-metabolizing risk score constructed in this study should be more specific for tobacco-carcinogen metabolism than any single variant of carcinogen-metabolizing enzymes. We also evaluated the interaction of carcinogen-metabolizing risk score with red meat intake, NSAID use, alcohol intake, BMI and physical activity, and found no interactions between these factors and polyp risk. Therefore, it is unlikely that potential pleiotropic effects of certain genes could appreciably affect the results from this study. Finally, based on the knowledge of carcinogen-metabolizing patterns, the carcinogen-metabolizing risk score derived from this study should affect the internal effective dose of carcinogen exposure and thus can further define internal-exposure status for people with the same external exposure level of tobacco carcinogen. Interestingly, the association of cancer risk with cigarette smoking (or aromatic amines) and carcinogen-metabolizing enzymes is among the examples provided by Smith (43) and Thomas (44) for Mendelian randomization analyses of gene–environment interactions.

We assigned a carcinogen-metabolizing score of '0', '1' or '2' for genotypes carrying zero, one or two risk alleles, respectively. Ideally, this score should be constructed by taking into consideration the effect size of each isozyme in the pathway of tobacco-carcinogen metabolism. However, because *in vitro* experiments to assess the function and effect size of these isozymes were conducted under various conditions, it would be difficult to use these data to construct a genetic score. To avoid over-fitting the data, we did not use the OR for each genetic variant as the weight to construct the genetic score. However, this simple approach allowed us to identify significant interactions. If we could take into consideration the effect size of each isozyme in construction of the carcinogen-metabolizing score, we expect that a stronger interaction between genetic factors and cigarette smoking might be observed.

This study focused on precursors for colorectal cancer, that is colorectal polyps, thus eliminating possible survival bias commonly encountered in cancer case–control studies as a result of failure to recruit patients with short survival time. Because these polyps were benign lesions, recall bias and any subsequent lifestyle change following polyp diagnosis are unlikely to be substantial. Other important strengths of this study are the use of colonoscopy to define patient groups and large sample size. The majority of study participants (87.8%) were recruited prior to colonoscopy and thus prior to polyp diagnosis, which reduces possible selection bias. Exclusion of participants recruited after colonoscopy (n = 894) did not appreciably change the associations observed. Response rates are not optimal in this study, which could introduce selection bias. However, as discussed previously, these potential biases can be reduced in our study through the use of Mendelian randomization analysis. Because we did not have data regarding internal exposure of tobacco carcinogens, the internal exposure level in our study was inferred using data from exposure assessment and the carcinogen-metabolizing score.

In summary, our findings suggest that genetic variants in carcinogen-metabolizing enzymes may modify the association of cigarette smoking with colorectal polyp risk. We expect additional functional variants in the carcinogen-metabolizing pathway will be identified in the future to improve the classification of participants into low- to high-carcinogen-metabolizing risk categories. In other words, the true synergistic effect of genetic factors and cigarette smoking could be stronger than what we observed in this study.

Supplementary material

Supplementary Appendices 1–4 can be found at http://carcin.oxfordjournals.org/

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