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Research Article

Genetic Variation in the TGF-β Signaling Pathway and Colon and Rectal Cancer Risk

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Abstract

Background: The TGF-β signaling pathway is an essential regulator of many cellular process involved in carcinogenesis. Smad proteins are central to the function of TGF-β signaling. In this study, we evaluated genetic variation in *TGFβ1*, *TGFβR1*, *Smad1*, *Smad2*, *Smad3*, and *Smad4* and risk of colon and rectal cancer.

Methods: Data are from a large case–control study of colon (n = 1,444 cases, 1,841 controls) and rectal (n = 754 cases, 856 controls) cancer participants with DNA.

Results: Both *TGFβ1* rs1800469 and rs4803455 were associated with colon cancer [odds ratio (OR) = 0.65 and 1.43, 95% CI = 0.51–0.84 and 1.18–1.73, respectively) but not rectal cancer. Likewise, 1 of 3 tagSNPs for *TGFβR1*, 2 of the 4 tagSNPs for *Smad2*, and 4 of 37 *Smad3* tagSNPs were associated with colon cancer. Fewer significant associations were observed for rectal cancer, with only 1 tagSNP in *Smad2* and 3 tagSNP in *Smad3* having 95% CIs excluding 1.0. Several *Smad3* tagSNPs were only associated with CpG island methylator phenotype. We observed several statistically significant interactions between genetic variation in the TGF-β signaling pathway and *NFκB1*, further illustrating its involvement in proposed mechanisms. In addition, we observed statistically significant interaction between *TGFβ1*, *TGFβR1*, and *Smad3* and cigarette smoking, aspirin use, and estrogen status for both colon and rectal cancers. Variation in *TGFβ1*, *TGFβR1*, and *Smad3* seemed to influence survival after diagnosis of colon and rectal cancer.

Conclusions: These findings provide further support for genetic variation in the TGF-β signaling pathway and risk of developing both colon and rectal cancers.

Impact: Insight into biological pathways is provided. *Cancer Epidemiol Biomarkers Prev; 20(1); 57–69. ©2011 AACR.*

Introduction

The TGF-β signaling pathway is an essential regulator of cellular proliferation, differentiation, apoptosis, and extracellular matrix remodeling in the cell (1). In addition, this signaling pathway is involved in angiogenesis and inflammation. It mediates intracellular actions of proinflammatory cytokines, including activation of nuclear factor-kappa B (NFκB; refs. 2, 3) and deficiency of TGF-β has been shown to lead to extensive inflammation (2). TGF-β ligands initiate their cellular effects by binding to cell surface receptors (1); type 1 receptors mediate their cellular effects through interaction with Smad proteins. Thus, Smads are key intracellular mediators of the transcriptional responses to TGF-β (4).

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Smad4 (DPC4) is inactivated in some colorectal cancers (CRC), and germline mutations of *Smad4* have been linked to familial juvenile polyposis families (5). *Smad2* has been identified as a TGF-β–responsive Smad that is a transcription factor involved in the regulation of cell growth and apoptosis. *Smad7* is also involved in inflammation-related pathways and has been shown to modulate TGF-β and Wnt signaling (6). Genetic variation in the *Smad7* gene on 8q21 has been identified through numerous genome-wide association studies (GWAS) as being associated with CRC (7). Like *Smad7*, *Smad2* and *Smad4* are located on 8q21. We previously reported on the replication of tagSNPs in the *Smad7* gene identified from GWAS in our population-based case–control study of colon cancer (8). We observed that rs12953717 was associated with a statistically significant increased risk of colon cancer [odds ratio (OR) = 1.38, 95% CI = 1.13–1.68; P linear trend < 0.01) for the TT genotype compared with the CC genotype whereas the CC genotype of the rs4939827 tagSNP was inversely associated with colon cancer (OR = 0.77, 95% CI = 0.64–0.93) relative to the TT genotype. In our study, associations seemed to be modified by use of aspirin (8).

There is growing support for the role of the TGF-β signaling pathway in the etiology of colon and rectal

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cancer. In this study, we evaluate genetic variation in *TGFβ1*, *TGFβR1*, *Smad1*, *Smad2*, *Smad3*, and *Smad4*. We evaluate how these genes interact with other potentially important genes in the pathway, including *Smad7*, *NFκB1*, and *IKBkB*, involved in inflammation-related mechanisms. Environmental factors that may operate in this pathway include estrogen, aspirin/ non-steroidal anti-inflammatory drugs (NSAID), and cigarette smoking that may lead to oxidative stress and increase the likelihood of inflammation (9). We evaluate the potential interactions between these factors and genetic variation in the TGF-β signaling pathway. In addition, we seek to confirm previous reports that genetic alterations in the TGF-β signaling pathway influences tumor markers such as microsatellite instability and epigenetic changes. We evaluate the hypothesis that the TGF-β signaling influences prognosis after diagnosis with cancer by comparing survival rates based on genetic variation in this pathway.

Methods

Two study populations are included in these analyses. The first study, a population-based case–control study of colon cancer, included cases ($n = 1,593$) and controls ($n = 1,994$) identified between October 1, 1991, and September 30, 1994 (10), living in the Twin Cities Metropolitan Area, Kaiser Permanente Medical Care Program of Northern California (KPMCP), and a 7-county area of Utah. The second study, with identical data collection methods, included cases with cancer of the rectosigmoid junction or rectum ($n = 790$) and controls ($n = 999$) who were identified between May 1997 and May 2001 in Utah and KPMCP (11). Eligible cases were between 30 and 79 years old at time of diagnosis, English speaking, mentally competent to complete the interview, had no previous history of CRC, and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Cohn's disease.

Controls were matched to cases by gender and by 5-year age groups. At KPMCP, controls were randomly selected from membership lists; in Utah, controls 65 years and older were randomly selected from the Health Care Financing Administration lists and controls younger than 65 years were randomly selected from driver's license lists. In Minnesota, controls were selected from driver's license and state-identification lists. Study details have been previously reported (12, 13).

Interview data collection

Data were collected by trained and certified interviewers using laptop computers. All interviews were audio-taped as previously described and reviewed for quality control purposes (14). The referent period for the study was 2 years prior to diagnosis for cases or selection for controls. Detailed information was collected on diet, physical activity, medical history, reproductive history, family history of cancer in first-degree relatives, regular use of aspirin and NSAIDs, and body size.

Tumor registry data

Tumor registry data were obtained to determine disease stage at diagnosis and months of survival after diagnosis. Disease stage was categorized by Surveillance, Epidemiology, and End Results (SEER) staging of local, regional, and distant disease as well as by the American Joint Committee on Cancer (AJCC) staging criteria. Local tumor registries provided information on patient follow-up including vital status, cause of death, and contributing cause of death. Survival months were calculated on the basis of month and year of diagnosis, and month and year of death, or date of last contact for those individuals who were still alive.

Tumor marker data

We have previously evaluated tumors for CpG island methylator phenotype (CIMP), microsatellite instability (MSI), *TP53* mutations, and *KRAS2* mutations (15–18) and were therefore able to evaluate genes in relation to tumors with specific characteristics or markers. Details for methods used to evaluate these epigenetic and genetic changes have been described in previous publications (15–18).

tagSNP selection and genotyping

tagSNPs were selected for genes *TGFβR1*, *Smad1*, *Smad2*, *Smad3*, and *Smad4*, using the following parameters: an $r^2 < 0.8$ defined LD blocks using a Caucasian LD map, minor allele frequency or MAF > 0.1, range = −1,500 bp from the initiation codon to +1,500 bp from the termination codon, and 1 SNP/LD bin. All markers were genotyped using a multiplexed bead array assay format based on GoldenGate chemistry (Illumina). A genotyping call rate of 99.85% was attained. Blinded internal replicates represented 4.4% of the sample set. The duplicate concordance rate was 100.00%.

For *TGFβ1*, candidate markers rs1800469 and rs4803455 were chosen on the basis of prevalent MAF and previous findings described in the literature (19); rs1800469 and rs4803455 were genotyped independently using a TaqMan assay from Applied Biosystems. Each 5-μL PCR reaction contained 20 ng of genomic DNA, primers, probes, and TaqMan Universal PCR Master Mix (containing AmpErase UNG, AmpliTaq Gold enzyme, dNTPs, and reaction buffer). PCR was carried out under the following conditions: 50°C for 2 minutes to activate UNG, 95°C for 10 minutes, followed by 40 cycles of 92°C for 15 seconds, and 60°C for 1 minute using 384 well duel block ABI 9700. Fluorescent endpoints of the TaqMan reactions were measured using a 7900HT sequence detection instrument.

Statistical methods

All statistical analyses were carried out using SAS version 9.2 (SAS Institute). We assessed ORs and 95% CIs in multiple logistic regression models for colon and rectal cancer separately. All SNPs were evaluated first by comparing the heterozygote and homozygote variants to the homozygote wild-type and subsequently assessing

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the likelihood of the dominant and recessive models of inheritance; the best fitting model is presented (20). *P* values from the unadjusted Max test were used to adjust for multiple comparisons of tagSNPs using the methods by Conneely and Boehnke (20, 21). Minimal adjustments were made for age, sex, race, and study center. Additional adjustments for BMI (kg/m^2), physical activity, use of aspirin or NSAIDs within 2 years of the referent period, and cigarette smoking status (ever or never regularly smoked) did not alter associations.

Stepwise regression models were used to identifying tagSNPs that contributed uniquely and most significantly to the overall fit of the model for colon and rectal as well as to identify potential confounding of tagSNPs within genes. Inclusion in the stepwise regression model was based on a score chi-square significance level of 0.05 whereas exclusion was determined on the basis of a Wald chi-square 0.05 significance level. Subsequent analysis for interaction was based both on tagSNPs remaining in the final stepwise model and those identified as being important independently.

We evaluate interaction between *TGFβ1* and its receptor and *Smad1*, *Smad2*, *Smad3*, *Smad4*, *Smad7*, *IKBκB*, and *NFκB1*. Possible interactions between SNPs and sex, age (30–64 or 65–79), recent aspirin or NSAID use, estrogen status, BMI (<25, 25–30, >30), and cigarette smoking were evaluated given the hypothesized mechanisms proposed for these genes. Associations between colon cancer and *Smad7*, *IKBkB*, and *NFκB1* have been previously reported (8; K. Curtin, R.K. Wolff, J.S. Herrick, R. Abo, M.L. Slattery, unpublished data). *P* values for interaction were determined by comparing a full model including an ordinal multiplicative interaction term to a reduced model without an interaction term using a likelihood ratio test; a categorical model was used for *TGFβ1* rs4803455 and smoking and for *Smad2* rs1792689 and *TGFβR1* rs1571590. Haplotypes based on the SNPs being identified as significant for each gene were examined with both environmental and gene interactions but did not yield any more meaningful results than looking at the individual SNPs and therefore are excluded.

Tumors were defined by specific alterations detected; any *TP53* mutation, any *KRAS2* mutation, MSI+, or CIMP+ defined as at least 2 of 5 markers methylated. As the proportion of MSI+ tumors in the rectal cases was <3% (22), there was insufficient power to examine these tumor markers with genotype data. Population-based controls were used to assess associations for the population overall when examining multiple outcomes defined by tumor status. In addition to identifying variants that contributed to a given phenotype independently, a stepwise regression of all SNPs per gene was implemented in SAS, using the logistic procedure for each individual tumor type.

Time of survival was determined on the basis of date of diagnosis and date of last contact or death, truncated at 5 years, the time period which is most meaningful for assessment of impact with CRC. Associations between SNPs and risk of dying of CRC within 5 years from diagnosis were evaluated using Cox proportional hazards models to provide multivariate hazard rate ratios (HRR) and 95% CIs adjusted for age at diagnosis, study center, race, sex, AJCC stage, and tumor markers. HRRs were assessed for SNPs independently and using stepwise regression via the phreg procedure adjusting for other SNPs.

Results

Table 1 describes the genes and corresponding SNPs associated independently, through interaction, or with tumor markers. All SNPs were in Hardy–Weinberg equilibrium (HWE). SNPs that were independently associated with colon or rectal cancer overall are shown in Figure 1. As shown in the figure, the following associations were observed for colon cancer: OR = 1.25 (95% CI = 1.03–1.51) TT versus AA for *Smad2* rs1787199; OR = 1.33 (95% CI = 1.06–1.67) CC versus TT for *Smad2* rs4940086; OR = 0.68 (95% CI = 0.55–0.85) for AG/GG versus AA for *Smad3* rs12901071; OR = 0.69 (95% CI = 0.57–0.84) CC versus AA for *Smad3* rs1498506; OR = 0.76 (95% CI = 0.59–0.98) for AA versus GG/GA for *Smad3* rs7163381, adjusted for rs1498506; OR = 0.68 (95% CI = 0.47–0.97) CC versus GG/GC for *Smad3* rs2414937; OR = 0.65 (95% CI = 0.51–0.84) for AA versus GG for *TGFβ1* rs1800469; OR = 1.43 (95% CI = 1.18–1.73) for AA versus CC for *TGFβ1* rs4803455; OR = 0.85 (95% CI = 0.74–0.99) for TA/AA versus TT for *TGFBR1* rs6478974. After adjustment for multiple comparisons, *Smad3* rs1498506 and rs12901071 remained statistically significant (adjusted *P* values of 0.0.009 and 0.015, respectively). Because *TGFβ1* rs1800469 and rs4803455 were candidate SNPs, we did not adjust them for multiple comparisons.

The following associations were statistically significant for rectal cancer (Figure 1): OR = 0.78 (95% CI = 0.62–0.98) for CT/TT versus CC for *Smad2* rs1792689; and OR = 1.81 (95% CI = 1.12–2.91) for CC versus TT/TC for *Smad3* rs17293443. Although *Smad3* rs11071933 and rs1866317 were not statistically significant independently, after adjusting for rs17293443 and one another, risk estimates were 0.75 (95% CI = 0.61–0.93 and 1.28 (95% CI = 1.03–1.59) for the CG/GG versus CC genotypes, respectively.

For colon cancer, we observed a statistically significant interaction between *Smad3* rs3825977 and *TGFβ1* rs1800469 and between *Smad2* rs4940086, *Smad3* rs17293443, and *Smad7* rs4939827 with *TGFβ1* rs4803455 (Table 2). Statistically significant interactions also were observed between both *TGFBR1* rs6478974 and rs1571590 with *IKBkB* rs37473811 and with *NFκB1* rs4648110 (Table 2). Statistically significant gene–gene interactions also were identified for rectal cancer (Table 3). *TGFβ1* rs1800469 interacted with *Smad3* rs211860 and rs4147358 (Table 3); *TGFβ1* rs4803455 and *TGFβR1* rs105733710 interacted with *NFκB1* rs4648110 and rs13117745; *TGFβR1* rs1571590 interacted significantly with *Smad2* rs1792689.

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Table 1. Summary of SNPs

Gene	Alias	Location	SNP	Major/Minor allele	MAF[a]	Colon		Rectal	
						Heterozygote OR (95% CI)	Homozygote rare OR (95% CI)	Heterozygote OR (95% CI)	Homozygote rare OR (95% CI)
Smad2	MAD2	18q21.1	rs1787199	A/T	0.46	1.08 (0.92–1.26)	1.24 (1.03–1.51)	0.85 (0.69–1.05)[b]	
	MADH2		rs1792689	C/T	0.13	0.96 (0.82–1.12)	1.30 (0.79–2.13)	0.78 (0.62–0.98)[b]	
	JV18		rs4940086	T/C	0.33	1.08 (0.94–1.25)	1.33 (1.06–1.66)	1.00 (0.81–1.23)	1.05 (0.77–1.42)
Smad3	MAD3	15q22.33	rs750766	G/A	0.48	0.98 (0.84–1.15)	0.93 (0.77–1.13)	1.09 (0.89–1.34)[b]	
	MADH3		rs893473	C/T	0.17	0.97 (0.84–1.13)	0.99 (0.69–1.40)		1.09 (0.73–1.62)[c]
	JV15-2		rs991157	G/A	0.3		0.93 (0.73–1.18)[c]		1.11 (0.79–1.55)[c]
			rs1498506	A/C	0.48	0.87 (0.75–1.02)	0.69 (0.57–0.84)	1.10 (0.88–1.38)	0.96 (0.72–1.26)
			rs1866317	C/G	0.11	0.98 (0.83–1.16)	0.92 (0.48–1.76)	1.12 (0.88–1.42)	1.65 (0.77–3.54)
			rs2118610	G/A	0.45	1.11 (0.95–1.29)	0.94 (0.78–1.14)	1.02 (0.82–1.27)	1.02 (0.77–1.36)
			rs2118611	A/G	0.2	1.03 (0.90–1.19)[b]		0.89 (0.73–1.09)[b]	
			rs2414937	G/C	0.2		0.68 (0.47–0.97)[c]	1.01 (0.82–1.24)	1.02 (0.63–1.67)
			rs3743343	T/C	0.24	1.10 (0.95–1.26)	1.15 (0.87–1.52)	0.97 (0.79–1.19)	1.10 (0.77–1.58)
			rs3825977	C/T	0.19	0.95 (0.82–1.11)	1.01 (0.72–1.42)	0.96 (0.78–1.18)	0.76 (0.47–1.24)
			rs4147358	C/A	0.22	1.08 (0.93–1.24)	0.99 (0.73–1.33)	1.03 (0.84–1.27)	0.89 (0.60–1.33)
			rs4776892	A/T	0.18	1.02 (0.89–1.18)[b]		0.94 (0.76–1.16)	1.14 (0.69–1.88)
			rs7163381	G/A	0.26		0.76 (0.59–0.98)[c]		1.11 (0.79–1.56)[c]
			rs7176870	A/G	0.43	1.08 (0.93–1.24)[b]		1.16 (0.94–1.42)[b]	
			rs11071933	C/G	0.33	1.04 (0.90–1.20)	0.94 (0.75–1.16)	0.84 (0.69–1.03)[b]	
			rs12901071	A/G	0.34		0.68 (0.55–0.85)[c]		0.80 (0.57–1.10)[c]
			rs17293443	T/C	0.22		0.84 (0.60–1.18)[c]		1.81 (1.12–2.91)[c]
Smad4	DPC4	18q21.1	rs10502913	G/A	0.24	1.03 (0.89–1.19)	0.81 (0.61–1.08)	1.02 (0.83–1.25)	1.09 (0.72–1.67)
	MADH4								
TGFβ1	TGFB	19q13.1	rs1800469	G/A	0.31	0.89 (0.78–1.03)	0.65 (0.51–0.84)	1.02 (0.84–1.23)[b]	
			rs4803455	C/A	0.48	1.25 (1.06–1.47)	1.43 (1.18–1.73)	1.06 (0.84–1.32)	1.04 (0.79–1.35)
TGFβR1	ALK-5	9q22	rs1571590	A/G	0.2	0.95 (0.82–1.10)	1.39 (0.98–1.96)	0.91 (0.74–1.12)	1.42 (0.85–2.39)
	SKR4		rs6478974	T/A	0.49	0.85 (0.74–0.99)[b]		0.85 (0.69–1.05)[b]	
	LDS1A		rs10733710	G/A	0.2	1.07 (0.93–1.23)[b]		1.17 (0.96–1.43)[b]	
	AAT5								

[a]MAF based on White control population.
[b]Dominant model.
[c]Recessive model.

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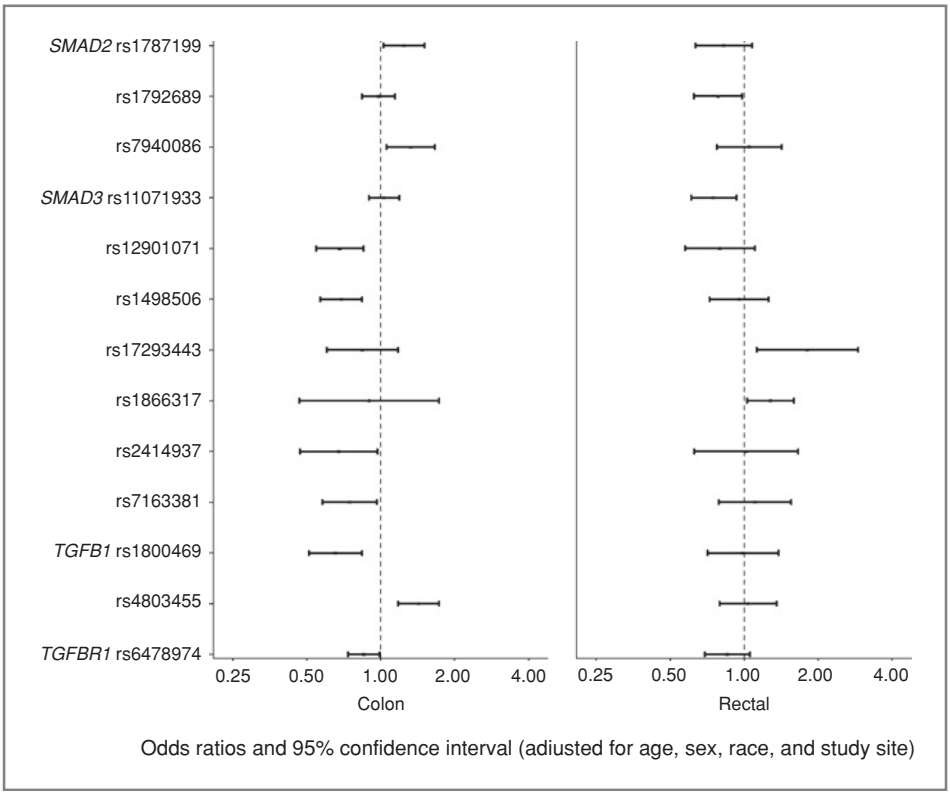

Figure 1. Associations between SNPs in the TGF-β signaling pathway and colon and rectal cancer.

Several variants within the TGF-β signaling pathway interacted with lifestyle factors hypothesized as influencing this pathway. Statistically significant interactions with cigarette smoking and colon cancer were observed for *TGFβ1* rs4803455, *TGFβR1* 10733710, and rs1571590 (Table 4). As previously noted, the AA genotype of *TGFβ1* rs4803455 increased risk of colon cancer overall, but the increase in risk was especially dramatic among recent smokers (OR = 2.09, 95% CI = 1.47–2.96). The GG genotype of *TGFβR1* rs1571590 was associated with increased colon cancer risk among nonsmokers/former smokers while there was a trend toward reduced risk among recent cigarette smokers for the same genotype. The A allele of *TGFβ1* rs1800469 was observed as increasing rectal cancer risk among recent smokers.

The *TGFβR1* rs6478974 A allele was associated with reduced risk of colon cancer among those who recently used aspirin/NSAIDs and had no effect among non–aspirin/NSAID users (Table 4). *Smad3* rs3743343 interacted significantly with aspirin/NSAIDs for both colon and rectal cancers, although the direction of the association was different for these cancer sites. Statistically significant interactions were observed for *Smad3* rs7173811 and aspirin/NSAIDs for colon cancer and both *Smad3* rs7163381 and rs11071933 and rectal cancer. Among these SNPs, those who had the variant allele were at increased risk if they did not use aspirin/NSAIDs regularly but were at significantly reduced risk if they used aspirin/NSAIDs regularly.

Among women recently exposed to estrogen, the A allele of *TGFβ1* rs1800469 was associated with a reduced risk of colon cancer and the C allele of rs4803455 was associated with a decreased risk of rectal cancer (Table 4). Likewise, both variants of *Smad4*, rs10502913 and rs8096092, were associated with increased risk of rectal cancer among men while reducing risk among women.

Unique sets of *Smad2*, *Smad3*, *TGFβ1*, and *TGFβR1* SNPs were associated with tumor phenotypes for colon and rectal cancer (Table 5). Among colon cancer cases, the risk of a CIMP+ tumor was associated with both *Smad2* and *Smad3*. *TGFβ1* rs1800469 was associated with a decreased risk for all colon tumor phenotypes except CIMP+, although not associated with rectal molecular phenotype. *TP53*-mutated colon tumors were associated with *Smad2* rs4940086 and *Smad3* rs7176870. MSI+ colon tumors were associated with *Smad2* rs1792689 and rs1787199 and *Smad3* rs12901071 and rs731874. For rectal cancer, *Smad3* rs893473 was associated with an increased likelihood of a CIMP+ tumor (OR = 3.6, 95% CI = 1.62–798) for the TT genotype relative to CC/CT; rs991157 AA versus GG/GA was associated with a statistically significant increased risk of a *KRAS2*-mutated tumor (OR = 1.63, 95% CI = 1.03–2.79). The *TGFβR1* rs10733710 GA/AA genotype was associated with increased risk for both CIMP+ tumors and *TP53*-mutated tumors.

Variations in *TGFβ1*, *Smad1*, *Smad2*, and *Smad4* were not associated with survival after diagnosis (data not

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Table 2. Interaction between variants in the TGF-β signaling pathway and *NFκB1* and *IKBκB* and risk of colon cancer[a]

	Controls, *n*	Cases, *n*	OR	95% CI	Controls, *n*	Cases, *n*	OR	95% CI	Controls, *n*	Cases, *n*	OR	95% CI
	***TGFβ1* rs1800469**											
	GG				**GA**				**AA**			
Smad3 rs3825977												
CC	610	521	1.00		542	419	0.92	0.77 1.09	116	80	0.80	0.58 1.08
CT	277	230	0.99	0.80 1.23	259	201	0.90	0.72 1.12	68	35	0.57	0.37 0.88
TT	30	43	1.67	1.03 2.70	35	19	0.64	0.36 1.14	13	2	0.17	0.04 0.75
$P_{interaction}$			<0.01									
	***TGFβ1* rs4803455**											
	CC				**CA**				**AA**			
Smad2 rs4940086												
TT	232	152	1.00		465	360	1.21	0.94–1.55	207	170	1.27	0.95–1.70
TC	228	162	1.10	0.83–1.47	425	335	1.24	0.96–1.59	196	180	1.46	1.09–1.95
CC	58	26	0.71	0.43–1.18	105	113	1.71	1.22–2.39	29	50	2.72	1.64–4.49
$P_{interaction}$			0.02									
Smad3 rs17293443												
TT/TC	485	330	1.00		947	779	1.23	1.04–1.45	420	380	1.35	1.11–1.64
CC	33	10	0.46	0.22–0.94	48	29	0.93	0.57–1.51	11	20	2.92	1.38–6.19
$P_{interaction}$			<0.01									
Smad7 rs4939827												
TT	115	106	1.00		255	225	0.99	0.72–1.37	123	110	0.99	0.69–1.44
TC/CC	403	233	0.63	0.46–0.86	738	582	0.87	0.65–1.16	309	290	1.04	0.76–1.42
$P_{interaction}$			0.02									
	***TGFβR1* rs6478974**											
	CC				**CA**				**AA**			
IKBκB rs3747811												
TT	148	155	1.00		255	208	0.78	0.58–1.05	124	84	0.66	0.46–0.95
TA	270	219	0.78	0.58–1.04	472	352	0.72	0.55–0.95	250	171	0.67	0.49–0.90
AA	115	104	0.87	0.61–1.24	239	171	0.69	0.51–0.94	83	90	1.04	0.71–1.52
$P_{interaction}$			0.04									
NFκB1 rs4648110												
TT	346	289	1.00		615	474	0.93	0.76–1.14	282	233	1.01	0.80–1.28
TA	163	175	1.29	0.99–1.68	311	234	0.91	0.72–1.15	156	105	0.82	0.61–1.10
AA	24	14	0.71	0.36–1.40	40	23	0.69	0.40–1.18	19	7	0.47	0.20–1.15
$P_{interaction}$			0.04									
	***TGFβR1* rs1571590**											
	AA				**AG**				**GG**			
IKBκB rs3747811[b]												
TT	347	268	1.00		166	156	1.24	0.94–1.62	14	23	2.24	1.13–4.44
TA	640	500	1.03	0.85–1.26	317	209	0.86	0.68–1.09	35	33	1.25	0.75–2.06
AA	273	239	1.14	0.90–1.44	147	111	1.01	0.75–1.36	17	16	1.24	0.61–2.51
$P_{interaction}$			0.04									

[a]Associations adjusted for age, sex, center and race.

[b]Similar associations were observed for *NFκB1* rs13117745 (C > T), $P_{interaction} = 0.02$.

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Table 3. Associations between variants in the TGF-β signaling pathway and *NFκB1 and Smad2 and Smad3* and rectal cancer risk[a]

	Controls, *n*	Cases, *n*	OR	95% CI	Controls, *n*	Cases, *n*	OR	95% CI	Controls, *n*	Cases, *n*	OR	95% CI
	***TGFβ1* rs1800469**											
	GG				**GA**				**AA**			
Smad3 rs2118610[b]												
GG	158	119	1.00		140	104	0.99	0.70–1.40	23	35	1.95	1.09–3.48
GA	224	166	1.03	0.75–1.40	178	156	1.18	0.86–1.63	52	35	0.91	0.56–1.49
AA	83	72	1.22	0.82–1.81	76	61	1.11	0.73–1.68	19	4	0.29	0.10–0.88
$P_{interaction}$			0.01									
Smad3 rs4147358[c]												
CC	254	204	1.00		220	181	1	0.76–1.31	63	31	0.58	0.36–0.93
CA	184	137	0.89	0.89–1.19	138	115	0.99	0.72–1.35	26	34	1.59	0.92–2.74
AA	27	16	0.67	0.35–1.28	36	25	0.83	0.48–1.43	5	9	1.96	0.64–6.03
$P_{interaction}$			<0.01									
	***TGFβ1* rs4803455**											
	CC				**CA**				**AA**			
NFκB1 rs13117745[d]												
CC	201	130	1.00		339	274	1.25	0.95–1.65	155	132	1.35	0.98–1.86
CT/TT	52	66	2.00	1.30–3.06	142	105	1.18	0.84–1.65	69	44	1.00	0.64–1.55
$P_{interaction}$			<0.01									
	***TGFβR1* rs10733710**											
	GG				**GA/AA**							
NFκB1 rs4648110[e]												
TT	405	270	1.00		207	201	1.45	1.13–1.86				
TA/AA	210	184	1.33	1.04–1.72	136	98	1.08	0.80–1.47				
$P_{interaction}$			<0.01									
	***TGFβR1* rs1571590**											
	AA				**AG**				**GG**			
Smad2 rs1792689												
CC	455	379	1.00		241	183	0.91	0.72–1.16	15	28	2.42	1.27–4.61
CT/TT	156	112	0.84	0.63–1.11	78	48	0.75	0.51–1.11	14	4	0.32	0.10–0.99
$P_{interaction}$			0.003									

[a]Association adjusted for age, sex, race and center.
[b]Similar associations were observed for *SMAD3* rs991157 (G > A), $P_{interaction} < 0.01$.
[c]Similar associations were observed for *SMAD3* rs745103 (T > A), $P_{interaction} < 0.01$.
[d]Similar associations were observed for *NFκB1* rs4648110 (T > A), $P_{interaction} = 0.02$.
[d]Similar associations were observed for *NFκB1* rs13117745 (C > T), $P_{interaction} = 0.01$.

shown in table). Four SNPs were associated with colon cancer survival: *TGFβR1* rs10733710 GA/AA versus GG (HRR = 0.73, 95% CI = 0.57–0.95); and 3 *Smad3* SNPs, rs11639295 TT versus CC/CT (HRR = 0.46, 95% CI = 0.27–0.80); rs12708492 CT/TT versus CC (HRR = 1.78, 95% CI = 1.27–2.50), and rs2414937 CC versus GG (HRR = 2.54, 95% CI = 1.29–3.95). For rectal cancer, 4 SNPs also were associated with survival, although the associated SNPs were different from those that were associated with colon cancer. For rectal cancer, the associations were as follows: *TGFβR1* rs6478974 AA versus TT genotype (HRR = 1.73, 95% CI = 1.08–2.78) and rs1571590 AG/GG versus AA genotype (HRR = 0.64, 95% CI = 0.43–0.95); *Smad3* rs12904944 GA/AA versus GG (HRR = 1.45,

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Table 4. Interaction between genetic variants in the TGF-β signaling pathway and lifestyle factors and risk of colon and rectal cancer

	Controls, *n*	Cases, *n*	OR[a]	95% CI	Controls, *n*	Cases, *n*	OR[a]	95% CI
Colon cancer	Never smoker/Former smoker				Recent smoker			
TGFβ1 rs4803455								
CC	422	274	1.00		96	66	1.04	0.74–1.48
CA	815	650	1.25	1.04–1.50	180	155	1.31	1.01–1.71
AA	363	306	1.33	1.07–1.65	68	94	2.09	1.47–2.96
$P_{interaction}$			0.05					
TGFβR1 rs10733710								
GG	1,004	775	1.00		223	178	0.99	0.79–1.24
GA/AA	586	452	0.99	0.85–1.16	120	138	1.46	1.12–1.90
$P_{interaction}$			0.03					
TGFβR1 rs1571590								
AA	1,053	797	1.00		206	207	1.29	1.04–1.60
AG	505	376	0.99	0.84–1.17	125	101	1.04	(0.78–1.37)
GG	51	62	1.63	1.10–2.37	15	10	0.88	0.39–1.98
$P_{interaction}$			0.05					
Rectal cancer								
TGFβ1 rs1800469								
GG	385	295	1.00		80	61	0.97	0.67–1.40
GA/AA	419	305	0.93	0.75–1.15	69	87	1.58	1.11–2.24
$P_{interaction}$			0.03					
Colon cancer	No recent aspirin/NSAID use				Recent aspirin NSAID use			
TGFβR1 rs6478974								
TT	329	313	1.00		202	160	0.83	0.64–1.07
TA	554	502	0.95	0.78–1.16	401	223	0.59	0.47–0.74
AA	253	238	1.01	0.79–1.28	201	101	0.54	0.40–0.71
$P_{interaction}$			0.03					
Smad3 rs3743343								
TT	663	567	1.00		462	291	0.73	0.61–0.88
TC	402	401	1.15	0.96–1.38	295	177	0.70	0.56–0.87
CC	70	85	1.43	1.02–2.00	47	18	0.45	0.26–0.78
$P_{interaction}$			0.02					
Smad3 rs7173811								
CC	323	263	1.00		228	147	0.79	0.61–1.03
CT	549	520	1.15	0.94–1.41	378	235	0.77	0.61–0.96
TT	264	270	1.24	0.98–1.57	198	104	0.63	0.47–0.85
$P_{interaction}$			0.03					
Rectal cancer								
Smad3 rs3743343								
TT	272	268	1.00		245	137	0.57	0.44–0.75
TC	205	173	0.84	0.65–1.10	156	105	0.69	(0.51–0.93)
CC	44	36	0.81	0.50–1.30	27	29	1.03	0.59–1.80
$P_{interaction}$			0.01					
Smad3 rs7163381[c]								
GG	268	229	1.00		206	151	0.87	0.66–1.14
GA	219	198	1.04	0.80–1.35	176	96	0.63	0.46–0.86
AA	34	50	1.65	1.02–2.67	46	24	0.58	0.34–0.99
$P_{interaction}$			0.01					

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Table 4. Interaction between genetic variants in the TGF-β signaling pathway and lifestyle factors and risk of colon and rectal cancer (Cont'd)

	Controls, *n*	Cases, *n*	OR[a]	95% CI	Controls, *n*	Cases, *n*	OR[a]	95% CI
Colon cancer	No recent estrogen exposure				Recent estrogen exposure			
TGFβ1 rs1800469								
GG	253	209	1.00		653	579	0.78	0.59–1.03
GA	213	200	1.16	0.89–1.51	612	433	0.62	0.47–0.82
AA	53	39	0.85	0.54–1.34	141	77	0.47	0.32–0.68
$P_{interaction}$			0.03					
Rectal cancer								
TGFβ1 rs4803455								
CC	40	40	1.00		213	155	0.53	0.32–0.90
CA	84	76	0.89	0.52–1.53	397	303	0.57	0.34–0.94
AA	45	25	0.54	0.28–1.05	179	151	0.64	0.38–1.08
$P_{interaction}$			0.04					
Smad4 rs10502913[c]	Men				Women			
GG	318	245	1.00		248	197	1.04	0.81–1.33
GA	196	174	1.17	0.90–1.53	144	94	0.86	0.63–1.17
AA	26	32	1.57	0.91–2.72	25	12	0.64	0.31–1.30
$P_{interaction}$			0.02					

[a]Adjusted for age, center, race, and sex.
[b]Similar association observed for *Smad3* rs11071933; $P_{interaction} = 0.03$.
[c]Similar associations observed for *Smad4* rs8096092; $P_{interaction} = 0.02$.

95% CI = 1.03–2.04) and rs3825977 CT/TT versus CC genotype (HRR = 1.55, 95% CI = 1.10–2.18).

Discussion

The TGF-β signaling pathway is thought to play a critical role in the carcinogenic process because of its involvement in the regulation of cell growth, differentiation, proliferation, and apoptosis (23). TGF-β exerts its physiologic effect by activating its receptors. Once the TGF-β receptor complex is activated, intracellular signaling is initiated. The TGF-β receptor complex activates the Smad signaling pathway by directly phosphorlyating Smad2 and Smad3 that work in conjunction with Smad4 (24). In this study, genetic variation in *TGFβ1* was associated with an increased risk of colon cancer, but not rectal cancer. Our evaluation of genetic variation in TGF-β signaling pathway showed several variants associated with colon and rectal cancer, acting independently as well as modifying the effect of other genetic and lifestyle factors.

A major function of TGF-β is mediating intracellular actions of proinflammatory cytokines, including activation of NFκB (2, 3). Deficiency of TGF-β has been shown to lead to extensive inflammation (2). Inflammation status of the gut seems to play a critical role in the etiology of both colon and rectal cancers (25). Our data support the role of TGF-β in an inflammation-related pathway, given the interaction between genetic variants of *NFκB1* and *TGFβ1* and *TGFβR1* for both colon and rectal cancers. NFκB is an important nuclear transcription factor that regulates a large number of cytokines and is critical for the regulation of inflammation; increased transcription of NFκB can increase inflammation and angiogenesis as well as cell survival and growth (26). IkBκB is a key regulator of NFκB transcriptional activity (27); its proteins are inhibitors of NFκB (26). In addition to the interaction between other genes involved in the regulation of inflammation and variants in the TGF-β signaling pathway, we observed significant interaction with recent use of aspirin/NSAIDs and *TGFβR1* rs6478974 and risk of colon cancer, further supporting an inflammation-related mechanism.

It has been hypothesized that cigarette smoking can influence inflammation via enhanced oxidative stress. Furthermore, cigarette smoke has been shown to regulate the effect of various cytokines, including TGF-β (27–30). We observed statistically significant interaction between *TGFβ1* and *TGFβR1* variants and cigarette smoke and colon cancer, thus supporting this link in a population-based study. We also observed statistically significant interaction between estrogen and *TGFβ1* rs4803455. Estrogen has many physiologic properties and has been shown to influence both inflammation and insulin (31, 32).

One of the major mechanisms of TGF-β signaling is through a Smad-dependent pathway (6); Smad7

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Table 5. Associations between tumor molecular phenotype and *TGFβ* and *Smad* genes

		Controls, *n*	Cases, *n*	OR[a]	95% CI
Colon tumors			**CIMP+**		
Smad2 rs1787199	AA	601	64	1.00	
Note: Similar results for rs4940086	AT/TT	1,355	208	1.46	1.09–1.97
Smad3 rs2118611[b]	AA	1,226	152	1.00	
	AG/GG	729	120	1.87	1.26–2.79
Smad3 rs4776892	AA	1,288	175	1.00	
	AT/TT	667	97	0.63	0.42–0.95
			***KRAS2* mutation**		
TGFβ1 rs4803455	CC	526	74	1.00	
	CA/AA	1,457	280	1.40	1.06–1.85
TGFβ1 rs1800469	GG	932	187	1.00	
	GA/AA	1,046	166	0.78	0.62–0.98
			***TP53* mutation**		
Smad2 rs4940086	TT/TC	1,762	449	1.00	
	CC	194	67	1.38	1.02–1.86
Smad3 rs7176870	AA	644	146	1.00	
	AG/GG	1,311	371	1.28	1.03–1.59
TGFβ1 rs4803455	CC	526	111	1.00	
	CA	1,014	267	1.27	0.99–1.63
	AA	443	144	1.56	1.18–2.07
TGFβ1 rs1800469	GG	932	275	1.00	
	GA/AA	1,046	243	0.78	0.64–0.95
			MSI unstable		
Smad2 rs1792689	CC	1,477	132	1.00	
Note: Similar results for rs1787199	CT	448	45	1.12	0.79–1.60
	TT	31	8	2.85	1.28–6.36
Smad3 rs12901071[b]	AA/AG	1,716	174	1.00	
Note: Similar results for rs731874	GG	240	11	0.43	0.23–0.83
TGFβ1 rs1800469	GG	932	110	1.00	
	GA/AA	1,046	80	0.64	0.47–0.86
Rectal tumors			**CIMP+**		
Smad3 rs893473	CC/CT	899	49	1.00	
	TT	60	10	3.60	1.62–7.98
TGFβR1 rs10733710	GG	615	27	1.00	
	GA/AA	343	32	2.10	1.24–3.57
			***KRAS2* mutation**		
Smad3 (rs991157)	GG/GA	876	150	1.00	
	AA	83	23	1.69	1.03–2.79
			***TP53* mutation**		
Smad3 rs11071933[b]	CC	385	127	1.00	
	CG/GG	572	150	0.72	0.54–0.95
Smad3 rs750766	GG	304	70	1.00	
Note: Similar results for rs12102171 and rs7176870	GA/AA	653	207	1.49	1.09–2.04
TGFβR1 rs10733710	GG	615	155	1.00	
	GA/AA	343	105	1.40	1.06–1.84

[a]Adjusted for age, center, sex, and race.
[b]tagSNPs presented for this gene are adjusted for one another.

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promotes the anti-inflammatory action of the TGF-β signaling pathway (6). Thus, we evaluated how genetic variants between *TGFβ1* and *TGFβR1* were associated with *Smad2*, *Smad3*, *Smad4*, and *Smad7*. We have previously reported on independent associations between *Smad7* and colon cancer (8). In this article, we provide information on *Smad2*, *Smad3*, and *Smad4*, which have been hypothesized as important components of the TGF-β signaling pathway (34), as well as evaluate how *Smad7* interacts with other genes in the pathway. Both Smad2 and Smad3 showed independent associations with colon cancer; however, several variants also showed consistent associations with CIMP+ tumors. Smad has been associated with epigenetic silencing in other cancers (35). *Smad2* and *Smad7* interacted significantly with *TGFβ1* and *TGFβR1*, further supporting the importance of multiple elements of the TGF-β signaling pathway in the etiology of colon and rectal cancer.

Both *TGFβR1* and *Smad3* were associated with survival after diagnosis with colon and rectal cancer. We evaluated genetic variations in our candidate pathway because of its documented role in cell differentiation, metastasis, and survival (36–38). These associations were detected independent of stage at time of diagnosis and tumor characteristics. Although many SNPs were associated with survival, the ones of most importance often varied after diagnosis with colon versus rectal cancer. It is not readily clear why these differences were observed; however, many differences have been detected previously for colon and rectal cancer, suggesting different elements to their etiology and possible prognosis.

There are many strengths and limitations to this study. Others have evaluated polymorphisms in *TGFβ1* with CRC and have found some associations with some polymorphisms (39, 40). In our study, we were able to thoroughly evaluate this candidate pathway, using both tagSNP and haplotype analysis, looking at colon and rectal cancers separately and evaluating associations that may be unique to certain tumor molecular phenotypes. The data are extensive and allow us to evaluate interactions with hypothesized genes as well as with hypothesized lifestyle factors. This approach has enabled us to acquire a more comprehensive understanding of the TGF-β signaling pathway and colon and rectal cancer. Although the candidate pathway and specific genes were hypothesize *a priori* as being associated with colon and rectal cancer, the process of a thorough evaluation lead to many comparisons. Replication of these findings in other studies is therefore needed.

Our data suggest that the TGF-β signaling pathway in conjunction with Smad is an important component of colon and rectal cancer risk and survival after diagnosis. Environmental factors, such as smoking cigarettes and using aspirin/NSAIDs, modulate this risk. Also of importance is the finding that some of these genes preferentially influenced the development of CIMP+ tumors, providing additional information on the carcinogenic process. Support for these findings from other similar studies is necessary to verify these associations.

Appendix

Summary of All Genes and SNPs Assessed

Gene	Chromosome location	SNP	Region	MAF	Major/Minor allele	FDR HWE probability	Colon homozygote are OR	Rectal homozygote rare OR
Smad1	4q31	rs714195	Intronic	0.42	G/A	0.73	0.99 (0.80–1.21)	1.02 (0.74–1.39)
		rs6537355	5 upstream	0.12	A/G	0.88	1.35 (0.72–2.54)	0.93 (0.37–2.32)
		rs2118438	Intronic	0.19	G/A	0.61	1.11 (0.75–1.65)	1.29 (0.72–2.34)
		rs1016792	Intronic	0.19	T/C	1.00	1.00 (0.69–1.46)	0.90 (0.51–1.58)
		rs12505085	3 downstream	0.23	A/G	0.89	0.88 (0.65–1.20)	0.91 (0.57–1.47)
Smad2	18q21.1	rs1787199	Intronic	0.46	A/T	1.00	1.24 (1.03–1.51)	0.83 (0.63–1.08)
		rs1792658	Intronic	0.21	A/C	0.96	1.18 (0.88–1.58)	1.12 (0.72–1.74)
		rs1792689	Intronic	0.13	C/T	0.95	1.30 (0.79–2.13)	0.95 (0.41–2.22)
		rs4940086	Intronic	0.33	T/C	1.00	1.33 (1.06–1.66)	1.05 (0.77–1.42)
Smad3	15q22.33	rs731874	Intronic	0.28	G/A	1.00	1.06 (0.82–1.37)	0.94 (0.65–1.37)
		rs745103	Intronic	0.45	T/C	0.86	0.96 (0.79–1.16)	0.98 (0.75–1.29)
		rs750766	Unknown	0.48	G/A	1.00	0.93 (0.77–1.13)	1.06 (0.81–1.39)
		rs893473	Intronic	0.17	C/T	1.00	0.99 (0.69–1.40)	1.06 (0.71–1.59)
		rs991157	Intronic	0.30	G/A	1.00	0.96 (0.75–1.23)	1.07 (0.75–1.51)
		rs1470003	Intronic	0.48	G/C	0.96	0.95 (0.79–1.15)	1.19 (0.90–1.57)
		rs1498506	Intronic	0.48	A/C	1.00	0.69 (0.57–0.84)	0.96 (0.72–1.26)
		rs1866317	Unknown	0.11	C/G	1.00	0.92 (0.48–1.76)	1.65 (0.77–3.54)

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Gene	Chromosome location	SNP	Region	MAF	Major/Minor allele	FDR HWE probability	Colon homozygote are OR	Rectal homozygote rare OR
		rs1992215	Unknown	0.33	T/C	1.00	1.00 (0.80–1.25)	0.86 (0.62–1.21)
		rs2118610	Intronic	0.45	G/A	0.61	0.94 (0.78–1.14)	1.02 (0.77–1.36)
		rs2118611	Intronic	0.20	A/G	0.99	0.94 (0.66–1.34)	0.93 (0.62–1.42)
		rs2414937	Intronic	0.20	G/C	1.00	0.67 (0.47–0.97)	1.02 (0.63–1.67)
		rs3743343	3 UTR	0.24	T/C	1.00	1.15 (0.87–1.52)	1.10 (0.77–1.58)
		rs3784681	Intronic	0.29	G/C	0.96	0.91 (0.71–1.17)	0.79 (0.56–1.11)
		rs3825977	Intronic	0.19	C/T	1.00	1.01 (0.72–1.42)	0.76 (0.47–1.24)
		rs4147358	Intronic	0.22	C/A	0.96	0.99 (0.73–1.33)	0.89 (0.60–1.33)
		rs4601989	Intronic	0.24	C/T	0.68	0.81 (0.60–1.08)	0.66 (0.44–1.00)
		rs4776881	Intronic	0.44	T/C	1.00	1.07 (0.89–1.30)	1.21 (0.92–1.60)
		rs4776890	Intronic	0.40	T/G	0.96	0.97 (0.80–1.19)	0.96 (0.72–1.28)
		rs4776892	Intronic	0.18	A/T	0.45	1.00 (0.67–1.48)	1.14 (0.69–1.88)
		rs7163381	Intronic	0.26	G/A	1.00	0.79 (0.61–1.03)	1.06 (0.74–1.51)
		rs7173811	Intronic	0.47	C/T	0.96	1.06 (0.88–1.28)	0.96 (0.73–1.26)
		rs7176870	Intronic	0.43	A/G	1.00	1.06 (0.88–1.29)	1.19 (0.90–1.58)
		rs7181556	Intronic	0.24	C/T	0.99	0.91 (0.69–1.22)	0.75 (0.50–1.12)
		rs7183244	Intronic	0.39	C/T	0.84	1.00 (0.81–1.23)	1.04 (0.77–1.41)
		rs9972423	Intronic	0.37	T/A	1.00	0.97 (0.79–1.20)	1.27 (0.94–1.73)
		rs11071933	Intronic	0.33	C/G	1.00	0.94 (0.75–1.16)	0.92 (0.68–1.25)
		rs11637581	Intronic	0.28	C/T	0.95	0.98 (0.76–1.28)	1.11 (0.77–1.59)
		rs11639295	Intronic	0.31	C/T	1.00	0.88 (0.70–1.12)	0.83 (0.59–1.16)
		rs12102171	Intronic	0.17	C/T	0.86	0.90 (0.62–1.30)	0.84 (0.49–1.43)
		rs12708492	Intronic	0.48	C/T	1.00	1.02 (0.85–1.24)	1.10 (0.84–1.44)
		rs12901071	Intronic	0.34	A/G	0.68	0.67 (0.53–0.84)	0.85 (0.60–1.20)
		rs12904944	Intronic	0.34	G/A	1.00	0.81 (0.64–1.01)	1.07 (0.79–1.47)
		rs12907997	Intronic	0.50	C/T	1.00	0.95 (0.78–1.14)	0.91 (0.70–1.20)
		rs12915039	Intronic	0.24	A/C	1.00	1.01 (0.76–1.35)	1.08 (0.72–1.61)
		rs16950687	Intronic	0.28	A/G	1.00	0.93 (0.72–1.21)	1.22 (0.85–1.76)
		rs17293443	Intronic	0.22	T/C	0.92	0.85 (0.61–1.19)	1.74 (1.08–2.82)
Smad4	18q21.1	rs8096092	Intronic	0.38	C/A	0.68	1.00 (0.81–1.23)	1.17 (0.87–1.59)
		rs10502913	Intronic	0.24	G/A	0.74	0.81 (0.61–1.08)	1.09 (0.72–1.67)
Smad7	18q21.1	rs1316447	Intronic	0.19	C/T	1.00	0.86 (0.60–1.23)	0.88 (0.53–1.45)
		rs2337106	Intronic	0.47	C/G	1.00	0.88 (0.72–1.06)	1.11 (0.85–1.45)
		rs2337107	Intronic	0.41	G/A	0.99	1.12 (0.92–1.36)	0.97 (0.74–1.28)
		rs3736242	Intronic	0.22	G/A	1.00	0.94 (0.69–1.28)	1.33 (0.84–2.09)
		rs3764482	Intronic	0.19	C/T	1.00	1.25 (0.86–1.81)	0.60 (0.34–1.07)
		rs4464148	Intronic	0.31	T/C	1.00	1.06 (0.83–1.35)	0.76 (0.54–1.09)
		rs4939827	Intronic	0.49	T/C	1.00	0.79 (0.66–0.95)	0.95 (0.73–1.23)
		rs4939832	Intronic	0.24	A/G	1.00	1.00 (0.76–1.32)	1.29 (0.87–1.92)
		rs7238442	Intronic	0.46	T/C	0.82	1.12 (0.93–1.35)	0.92 (0.71–1.20)
		rs12456328	Intronic	0.13	C/T	1.00	0.81 (0.49–1.33)	1.16 (0.51–2.66)
		rs12953717	Intronic	0.42	C/T	1.00	1.36 (1.12–1.65)	0.90 (0.68–1.19)
TGFβ1	19q13.1	rs1800469	5 upstream	0.31	G/A	1.00	0.65 (0.51–0.84)	0.98 (0.71–1.38)
		rs4803455	Intronic	0.48	C/A	0.92	1.43 (1.18–1.73)	1.04 (0.79–1.35)
TGFβR1	9q22	rs1571590	Intronic	0.20	A/G	0.67	1.39 (0.98–1.96)	1.42 (0.85–2.39)
		rs6478974	Intronic	0.49	T/A	1.00	0.86 (0.71–1.04)	0.84 (0.63–1.10)
		rs10733710	Intronic	0.20	G/A	0.96	1.06 (0.77–1.46)	1.22 (0.78–1.91)

NOTE: MAF and FDR-adjusted Hardy-Weinberg Equilibrium (FDR HWE) based on White control population. ORs are adjusted for age, center, race, and sex.

Disclosure of Potential Conflicts of Interest

The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official view of the National Cancer Institute.

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