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Increased Risk of Colon Cancer Associated with a Genetic Polymorphism of *SMAD7*

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Abstract

Genome-wide association studies (GWAS) have identified *SMAD7* on 8q21 as being associated with colorectal cancer. We evaluated single nucleotide polymorphisms (SNP) in the *SMAD7* gene, including rs4939827, rs12953717, and rs4464148, previously identified from GWAS in a large population-based case-control study of colon cancer. We observed that rs12953717 was associated with a statistically significant increased risk of colon cancer [odds ratio, 1.38; 95% confidence intervals (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 SNP was inversely associated with colon cancer (0.77; 95% CI, 0.64–0.93) relative to the TT genotype. There were no significant differences in association for either of these polymorphisms when stratified by age, tumor site, sex, or family history. The odds ratios between *SMAD7* and colon cancer among individuals reporting recent aspirin/nonsteroidal anti-inflammatory drug use was 0.60 (95% CI, 0.43–0.85) for the CC genotype of the rs4939827 polymorphism and 1.69 (95% CI, 1.20–2.38) for the TT genotype of the rs1295371 polymorphism. This result compares to odds ratios of 0.86 (95% CI, 0.68–1.09) for rs4939827 and 1.22 (95% CI, 0.96–1.56) among individuals who did not use aspirin/nonsteroidal anti-inflammatory drugs. An assessment of *SMAD7* genotypes with tumor markers did not reveal any significant differences by *KRAS2*, *TP53*, CpG island methylator phenotype, or microsatellite instability status. No significant associations were observed for the rs4464148 SNP or other SNPs evaluated in the *SMAD7*. These results corroborate the findings of GWAS in colon cancer pointing to *SMAD7* and reinforce interest in SNPs in this gene.

Introduction

Genome-wide association studies (GWAS) have detected associations between various loci and colon cancer. 8q24, 8q23, 11q23, 3q21, and *SMAD7* on 18q21 have all been identified as potentially involved in colon cancer etiology (1–6). Of these loci on chromosomal regions of 8q21, biological rationale exists for an association between *SMAD7* and colorectal cancer

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(CRC). *SMAD7* is involved in inflammation-related pathways and has been shown to modulate transforming growth factor-β (TGF-β) and Wnt signaling (7), which are central to the development of colon tumors. Three single nucleotide polymorphisms (SNP) of the *SMAD7* gene, rs4939827, rs12953717, and rs4464148, were identified in the GWAS by Broderick and colleagues for both adenomas and cancers (2). Those carrying the rs4939827 homozygote variant genotype showed a 27% reduced risk of CRC [95% confidence intervals (CI), 0.66–0.80], rs12953717 was associated with a 37% increased risk for those with the homozygote variant genotype (95% CI, 1.25–1.50), and rs4464148 was associated with a 35% increased risk for the homozygote variant genotype (95% CI, 1.20–1.51). These three SNPs map to the same linkage disequilibrium block within intron 3 of the *SMAD7* gene. Another GWAS conducted among individuals with a family history of CRC observed that the rs4939827 *SMAD7* SNP was inversely associated with CRC (2). In contrast to other studies, the study by Tenesa and colleagues (1) found a statistically consistent 20% increased risk of CRC for the rs4939827 *SMAD7* variant allele, rather than a 27% reduction in risk reported by others; it is possible that these results reflect different variant alleles in the population studied, given that the minor allele frequency was close to 0.5 (and two GWAS did not report the actual genotypes but instead OR_{hom} or OR_{het}). The same group reported an 18% (95% CI, 1.12–1.23) increase per T allele of variant rs12953717.

Using data from a large multi-center study of colon cancer, we looked to confirm those associations as well as to determine other factors that might influence the *SMAD7* SNPs and colon cancer association. Factors evaluated include age, tumor site, sex, family history of CRC in first-degree relatives, and recent use of aspirin/nonsteroidal anti-inflammatory drugs (NSAID) given that *SMAD7* seems to be involved in inflammation-related mechanisms.

Materials and Methods

Data for the study came from colon cancer case-control studies conducted in Utah, the Northern California Kaiser Permanente Medical Care Program, and the Twin Cities Metropolitan area of Minnesota. Eligibility included being between 30 and 79 y of age at the time of diagnosis, English-speaking, mentally competent to complete the interview, no previous history of CRC, and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease. Controls were frequency-matched to cases by sex and by 5-y age groups. At the Kaiser Permanente Medical Care Program, controls were randomly selected from membership lists. In Utah, controls 65 y and older were randomly selected from lists provided by the Centers for Medicare and Medicaid Services (formerly Heath Care Finance Administration) and controls younger than 65 were randomly selected from driver's license lists. In Minnesota, controls were randomly selected from driver's license lists. Study eligibility and recruitment details of the study have been published previously (8,9). Roughly 91% of the population was non-Hispanic white.

Trained and certified interviewers collected diet and lifestyle data as previously outlined (10, 11). The referent year for the study was the calendar year ~2 y prior to the date of diagnosis (cases) or selection (controls). Information was collected on demographic factors such as age, sex, and study center, diet, physical activity, aspirin and nonsteroidal drug use, body size, and other lifestyle factors including medical, family, and reproductive history.

DNA was extracted from blood drawn from study participants; these analyses were limited to those individuals who provided a blood sample, which represented roughly 85% of those requested to provide a sample. Genotyping of the 11 *SMAD7* SNPs, rs4939827, rs4464148, rs2337107, rs7238442, rs12953717, rs12456328, rs2337106, rs4939832, rs3764482, rs1316447, and rs3736242 were performed in multiplex using GoldenGate assays (Illumina; see Appendix), marker rs4939827 failed on the Illumina platform and thus was subsequently

genotyped using a TaqMan-based assay. Twenty nanograms of genomic DNA from each individual were subjected to the TaqMan assay (ABI) according to the methods of the manufacturer. Data were collected using an ABI 7900HT instrument. Intraplate and interplate replicates at a rate of ~5% were included on all plates and in all batches with no discordant genotypes in replicates. Polymorphisms were evaluated separately in both cases and controls and were found to be in Hardy-Weinberg equilibrium (Table 1).

We have previously evaluated tumors for CpG island methylator phenotype (CIMP), microsatellite instability (MSI), *TP53* mutations, and *KRAS2* mutations (12–15) and were therefore able to evaluate *SMAD7* genotypes with specific tumor markers. Details for methods used to evaluate these epigenetic and genetic changes have been described in previous publications (12–15).

SAS statistical package, version 9.1 (SAS Institute) was used to conduct the analyses. Multivariate logistic regression models were used to evaluate the associations between colon cancer and *SMAD7* genotypes. All logistic regression models were minimally adjusted for age at selection or diagnosis, study center, race or ethnicity, and sex because these were categorical study-matching variables. We evaluated associations adjusting for other colon cancer risk factors including body mass index (BMI; kg/m^2), long-term vigorous physical activity level, recent use of aspirin/NSAIDs (defined as use within the past 2 y), and dietary calcium and energy intake. Odds ratios (OR) and 95% CI were used to report associations obtained from the multivariate logistic regression models. Trend across genotype was assessed by comparing the log likelihood of a logistic regression model with the genotype as an ordered categorical variable to the log likelihood of a model without the genotype using a χ^2 test with 1 *df*.

Multivariate logistic regression models were also used to evaluate the joint association of colon cancer with the *SMAD7* polymorphism, age, sex, family history of CRC among first-degree relatives, and use of aspirin/NSAIDs within the past 2 y. Proximal tumors were defined as those tumors located in the cecum through the transverse colon and distal tumors in the splenic flexure, descending, and sigmoid colon. Effect modification between genotypes and exposure variables was evaluated by a likelihood ratio test for a multiplicative interaction term in the logistic regression model. Multinomial logistic regression models were used to evaluate the associations of tumor characteristics and *SMAD7* polymorphisms. Effect modification between tumor characteristics and *SMAD7* polymorphisms was evaluated by a likelihood ratio test of a case-case only logistic regression model with the variable of interest to a model without the variable using a χ^2 test with 2 *df*.

Results

The minor allele frequency for rs4939827 was 49%, 42% for rs12953717 (T allele), and 30% for the rs4464148 (C allele) in this population (Table 1). The r^2 value between rs4939827 and 12953717 was 0.59 (data not shown in table). The rs12953717 *SMAD7* variant was associated with a statistically significant increased risk of colon cancer in this population (OR, 1.38; 95% CI, 1.13–1.68), whereas rs4939827 was associated with a significant inverse association with colon cancer (OR, 0.77; 95% CI, 0.64–0.93; Table 2); a significant linear trend was observed for both SNPs (*P* linear trend < 0.01). The rs4464148 variant along with the other SNPs assessed was not associated with altered risk of colon cancer. We did not detect stronger associations by combined genotype or by haplotype.

Further evaluation of both variants showed no statistically significant differences in association with colon cancer according to tumor site, sex, age, and family history of CRC for either the rs4939827 or the rs12953717 variants (Table 3). There was a significant linear trend across both polymorphisms of altered risk among those who reported recent use of aspirin/NSAIDs

(OR, 0.60; 95% CI, 0.43–0.85 for rs4939827 and OR, 1.69; 95% CI, 1.20–2.38 for rs1295371) but not among those who did not recently use aspirin/NSAIDs, although the difference between the two groups was not statistically significant (*P* interaction, 0.08 and 0.10, respectively).

We evaluated associations between the *SMAD7* variant and tumor markers (Table 4). Although the strongest associations for any tumor marker were observed for MSI tumors (OR, 1.58; 95% CI, 1.03–2.43 comparing the TT versus the CC genotypes for rs1295371 and OR, 0.68; 95% CI, 0.44–1.04 comparing the CC to TT genotypes of rs4939827), for the most part, differences were minimal when looking at CIMP positive versus CIMP negative, MSS versus MSI, *KRAS2* wild-type versus mutated tumors, and *TP53* wild-type versus mutated tumors.

Discussion

Consistent with the GWAS by Broderick and colleagues (2), we observed a statistically significant association between both the rs4939827 the rs12953717 *SMAD7* polymorphisms and colon cancer; however, we did not observe a similar association for the rs4464148 variant and risk of colon cancer in this large multi-centered population-based study. The strength of associations reported here are similar to those previously reported.

Because of the extensive data available from this study, we have been able to further define associations based on demographic and tumor characteristics of the population. We observed slightly stronger associations for men, distal tumors, and older individuals, none of which significantly varied according to the categories assessed. Although not significant, there was a suggestion of difference in association by aspirin/NSAID use for both the rs4939827 and the rs12953717 polymorphisms. Because *SMAD7* is plausibly involved in an inflammation-related pathway through its regulation of TGF-β, it is logical that exogenous factors which regulate inflammation might modulate the association between *SMAD7* and CRC. More powerful studies should assess this association to see if there is an interaction that we were underpowered to detect.

The potential importance of *SMAD7* in the etiology of CRC is supported from several avenues of research other than the GWAS. TGF-β mediates the intracellular actions of proinflammatory cytokines, including the activation of nuclear factor-Kβ (16,17). Deficiency of TGF-β has been shown to lead to extensive inflammation (16). SMAD7 promotes the anti-inflammatory action of the TGF-β signaling pathway (7). However, SMAD7 has other mechanisms that are relevant to CRC. Studies in mice have shown that the *SMAD7* IVs2-21 variant was associated with type 2 diabetes (18). An insulin-related pathway has been proposed for CRC, on the other hand, the association with type 2 diabetes might also have an inflammation association because inflammation processes influence insulin-related pathways (19). Other studies have shown that SMAD7 degrades β-catenin signaling, altering the Wnt-signaling pathway which is a central element in CRC (20). The *SMAD7* genotype has been associated with survival after diagnosis with CRC (3).

The GWAS conducted by Tomlinson and colleagues evaluated associations among individuals with a family history of CRC (21,22). Although this design strategy was done to enrich the sample for identification of susceptibility alleles, studies have shown different risk factors for individuals with a family history and those without a family history (22). Many lifestyle risk factors have been shown to have a greater influence among those without a family history of CRC compared with those with a family history of CRC (2,22,23). Our examination of *SMAD7* according to family history casts further light on the interpretation and evaluation of GWAS that focus on those only with a family history of CRC.

Because of our extensive data set which includes not only demographics, diet, and lifestyle exposures, but also tumor markers, we were able to evaluate whether the previously identified

SMAD7 SNPs were associated with specific tumor types. Although slight variations existed according to tumor markers, we did not observe statistically significant differences according to any of the markers evaluated.

Whereas GWAS have included colon and rectal tumors together, our study examined only colon cancer. Several studies of both environmental and genetic factors suggest different etiologies for colon and rectal cancers (24–26). The study by Broderick did not detect differences by site, although it is unclear how they defined tumor site. We observed only slightly stronger nonsignificant associations for more distal tumors; the study by Curtin and colleagues (27) suggest significant associations for distal tumors only, suggesting that site might be a relevant factor when considering risk associated with the *SMAD7* variants.

Results from this study add to the growing body of knowledge that *SMAD7* is an important component of CRC development. While providing support for an association between *SMAD7* variants and risk of colon cancer, we have added to previous findings by examining these variants with lifestyle and demographic factors and tumor mutations. Further research on the functionality of *SMAD7* variants is needed to better understand the observed associations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

SMAD7 SNPs analyzed

	MAF	HWE, *P* Adjusted
SMAD7 (rs4939827)	0.49	
SMAD7 (rs4464148)	0.30	0.6251
SMAD7 (rs2337107)	0.43	0.4345
SMAD7 (rs7238442)	0.47	0.2215
SMAD7 (rs12953717)	0.42	0.9734
SMAD7 (rs12456328)	0.13	0.8662
SMAD7 (rs2337106)	0.46	0.6053
SMAD7 (rs4939832)	0.25	0.9563
SMAD7 (rs3764482)	0.18	0.9734
SMAD7 (rs1316447)	0.19	0.7406
SMAD7 (rs3736242)	0.23	0.8104

Abbreviations: HWE, Hardy-Weinberg equilibrium; MAF, minor allele frequency.

Table 2

Associations between rs4939827 and rs12953717 and risk of colon cancer

	Controls	Cases	
	n	*n*	OR* (95% CI)
rs4939827			
TT	503	457	1.00
TC	992	773	0.86 (0.73–1.01)
CC	492	360	0.77 (0.64–0.93)
P trend			<0.01
rs12953717			
CC	676	503	1.00
CT	928	754	1.09 (0.94–1.28)
TT	327	332	1.38 (1.13–1.68)
P trend			<0.01

*Risk estimates adjusted for age, center, race, sex, BMI, long-term activity level, average number of cigarettes per day, recent aspirin/NSAID use, dietary calcium, and energy intake.

Table 3

Stratified associations between rs4939827 and rs12953717 and risk of colon cancer

	Proximal			Distal		
	Controls, *n*	**Cases, *n***	**OR (95% CI)**	**Controls, *n***	**Cases, *n***	**OR (95% CI)**
rs4939827						
TT	503	206	1.00	503	218	1.00
TC	992	348	0.85 (0.69–1.04)	992	367	0.86 (0.70–1.05)
CC	492	172	0.82 (0.64–1.04)	492	164	0.73 (0.58–0.94)
P trend			0.11			<0.01
P heterogeneity*			0.72			
rs12953717						
CC	676	235	1.00	676	235	1.00
CT	928	346	1.07 (0.88–1.30)	928	355	1.10 (0.90–1.34)
TT	327	143	1.26 (0.98–1.62)	327	161	1.41 (1.10–1.81)
P trend			0.10			<0.01
P heterogeneity*			0.76			
	No family history			Family history		
rs4939827						
TT	454	377	1.00	49	80	1.00
TC	897	643	0.86 (0.72–1.02)	95	130	0.87 (0.54–1.39)
CC	450	312	0.79 (0.64–0.97)	42	48	0.71 (0.40–1.27)
P trend			0.02			0.25
P heterogeneity*			0.76			
rs12953717						
CC	619	424	1.00	57	79	1.00
CT	834	628	1.11 (0.94–1.30)	94	126	0.90 (0.56–1.44)
TT	295	276	1.40 (1.13–1.72)	32	56	1.20 (0.66–2.16)
P trend			<0.01			0.64
P heterogeneity*			0.57			
	No recent aspirin/NSAID use					
rs4939827						

	Proximal			Distal		
	Controls, *n*	**Cases, *n***	**OR (95% CI)**	**Controls, *n***	**Cases, *n***	**OR (95% CI)**
TT	300	297	1.00	201	158	1.00
TC	558	520	0.93 (0.76–1.14)	433	247	0.74 (0.56–0.97)
CC	306	267	0.86 (0.68–1.09)	184	90	0.60 (0.43–0.85)
P trend			0.22			<0.01
P heterogeneity*			0.08			
rs12953717						
CC	396	359	1.00	278	141	1.00
CT	535	503	1.02 (0.84–1.23)	391	245	1.27 (0.97–1.67)
TT	198	219	1.22 (0.96–1.56)	128	111	1.69 (1.20–2.38)
P trend			0.14			<0.01
P heterogeneity*			0.10			
			Men			
rs4939827						
TT	261	257	1.00	242	200	1.00
TC	535	430	0.84 (0.67–1.05)	457	343	0.87 (0.68–1.10)
CC	267	201	0.76 (0.59–0.99)	225	159	0.77 (0.58–1.02)
P trend			0.04			0.07
P heterogeneity*			0.97			
rs12953717						
CC	373	280	1.00	303	223	1.00
CT	484	413	1.10 (0.89–1.35)	444	341	1.09 (0.87–1.37)
TT	170	196	1.48 (1.13–1.92)	157	136	1.27 (0.95–1.71)
P trend			<0.01			0.12
P heterogeneity*			0.51			
			<65 y			
rs4939827						
TT	216	186	1.00	287	271	1.00
TC	371	314	1.01 (0.78–1.30)	621	459	0.77 (0.63–0.95)
CC	209	160	0.85 (0.63–1.14)	283	200	0.72 (0.56–0.93)
P trend			0.30			<0.01

	Proximal			Distal		
	Controls, *n*	**Cases, *n***	**OR (95% CI)**	**Controls, *n***	**Cases, *n***	**OR (95% CI)**
P heterogeneity*			0.36			
rs12953717						
CC	284	211	1.00	392	292	1.00
CT	346	313	1.22 (0.96–1.56)	582	441	1.01 (0.83–1.24)
TT	141	134	1.29 (0.95–1.75)	186	198	1.42 (1.10–1.84)
P trend			0.07			0.02
P heterogeneity*			0.86			

*Risk estimates adjusted for age, center, race, sex, BMI, long-term activity level, average number of cigarettes per day, recent aspirin/NSAID use, dietary calcium and energy intake.

Table 4

Associations between rs4939827 and rs1295371 and colon tumor mutations

	Controls *n*	Cases *n*	CIMP low *n*	Cases OR (95% CI)	CIMP high OR (95% CI)
rs4939827					
TT	503	216	1.00	76	1.00
TC	992	364	0.84 (0.68–1.03)	142	0.90 (0.67–1.23)
CC	492	172	0.77 (0.60–0.98)	61	0.77 (0.54–1.12)
P trend			0.04		0.15
P heterogeneity*			0.69		
rs12953717					
CC	676	241	1.00	82	1.00
CT	928	362	1.10 (0.91–1.34)	142	1.24 (0.93–1.67)
TT	327	153	1.34 (1.04–1.71)	55	1.41 (0.97–2.05)
P trend			0.03		0.05
P heterogeneity*			0.68		
			MSI stable		MSI unstable
rs4939827					
TT	503	277	1.00	58	1.00
TC	992	496	0.90 (0.74–1.08)	92	0.79 (0.56–1.12)
CC	492	234	0.83 (0.66–1.03)	40	0.68 (0.44–1.04)
P trend			0.09		0.07
P heterogeneity*			0.60		
rs12953717					
CC	676	325	1.00	55	1.00
CT	928	481	1.07 (0.90–1.28)	96	1.25 (0.88–1.77)
TT	327	199	1.28 (1.02–1.60)	42	1.58 (1.03–2.43)
P trend			0.04		0.04
P heterogeneity*			0.55		
			KRAS2 wild-type		*KRAS2* mutated
rs4939827					
TT	503	221	1.00	94	1.00

	Controls *n*	Cases *n*	CIMP low *n*	Cases OR (95% CI)	CIMP high OR (95% CI)
TC	992	370	0.84 (0.69–1.03)	175	0.92 (0.70–1.21)
CC	492	169	0.75 (0.59–0.96)	85	0.87 (0.63–1.20)
P trend			0.02		0.39
P heterogeneity*			0.74		
rs12953717					
CC	676	240	1.00	113	1.00
CT	928	367	1.10 (0.91–1.34)	170	1.08 (0.83–1.40)
TT	327	154	1.33 (1.04–1.70)	75	1.40 (1.01–1.94)
P trend			0.02		0.05
P heterogeneity*			0.87		
			TP53 wild-type		*TP53* mutated
rs4939827					
TT	503	174	1.00	144	1.00
TC	992	293	0.84 (0.67–1.04)	269	0.93 (0.74–1.17)
CC	492	152	0.85 (0.66–1.10)	113	0.77 (0.58–1.01)
P trend			0.17		0.07
P heterogeneity*			0.38		
rs12953717					
CC	676	209	1.00	161	1.00
CT	928	280	0.96 (0.78–1.19)	269	1.21 (0.97–1.51)
TT	327	133	1.33 (1.02–1.72)	97	1.26 (0.95–1.69)
P trend			0.07		0.07
P heterogeneity*			0.15		

*Risk estimates adjusted for age, center, race, sex, BMI, long-term activity level, average number of cigarettes per day, recent aspirin/NSAID use, dietary calcium and energy intake.