Short Communication

Transcription Factor 7–like 2 Polymorphism and Colon Cancer

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Abstract

Polymorphisms of the *transcription factor 7–like 2* (*TCF7L2*) gene have been associated with insulin sensitivity and diabetes, and the *TCF7L2* gene is involved in the Wnt/β-catenin signaling pathway, all factors thought to be important in the etiology of colon cancer. In this confirmatory study, we evaluated the rs7903146 *TCF7L2* polymorphism with colon cancer using previously collected data on 1,578 cases and 1,966 controls. We did not observe a statistically significant association between the rs7903146 polymorphisms and risk of colon cancer [odds ratio (OR), 1.12; 95% confidence interval (95% CI), 0.98-1.28] when evaluating the total population. We did, however, observe a statistically significant interaction between the rs7903146 *TCF7L2* polymorphism and recent use of aspirin/nonsteroidal anti-inflammatory drugs (NSAID; $P = 0.001$). Increased colon cancer risk associated with the T allele was restricted to those without recent use of aspirin/NSAIDs (OR, 1.65; 95% CI, 1.35-2.02, relative to recent aspirin users, i.e., use of aspirin/NSAIDS within the 2 years before diagnosis, with the CC genotype). Among individuals who reported recent use of aspirin/NSAIDs, the T allele reduced risk of colon cancer (OR, 0.78; 95% CI, 0.62-0.98) in a dose-response fashion (P for linear trend across genotypes = 0.03). These data suggest that colon cancer risk associated with the rs7903146 *TCF7L2* polymorphism is modified by use of aspirin/NSAIDs. (Cancer Epidemiol Biomarkers Prev 2008;17(4):978–82)

Introduction

The transcription factor 7–like 2 (*TCF7L2*) gene, also known as *TCF-4*, has been associated with insulin sensitivity and type 2 diabetes. Genome-wide association studies have identified the rs7903146 marker as being associated with type 2 diabetes (1). Follow-up studies have consistently shown that this marker is also predictive of developing type 2 diabetes (2). Studies of functionality have shown support for a dominant model, with the T allele being associated with impaired insulin secretion and low response to an oral glucose tolerance test (2). In addition to its functional role in insulin regulation, *TCF7L2* is involved in the *Wnt*/β-catenin signaling pathway (3), which is critical to proper functioning of the *APC* gene (4, 5). Thus, any cancer-related association with *TCF7L2* may stem from its roles either in insulin insensitivity or in the Wnt/β-catenin signaling pathway.

Although biologically plausible, few studies have examined associations between polymorphisms of the *TCF7L2* gene and cancer. There has been one report of an association with endometrial cancer (6) and another with familial breast cancer (7). Because of the well-documented role of the *APC* gene in development of colon cancer (5, 8), it is reasonable to hypothesize an association between *TCF7L2* and colon cancer. Findings by Folsom and colleagues using data from the Atherosclerosis Risk in Communities Study suggest that an association exists.[5] In their study, the TT genotype of the rs7903146 *TCF7L2* gene was associated with a >2-fold increased risk of colon cancer (hazard rate ratio, 2.15; 95% CI, 1.27-3.64).

Using data from a large multicenter study of colon cancer, we looked to confirm those associations as well as to determine other colorectal cancer risk factors that might interact with this pathway given their association with insulin-related factors. Factors evaluated include

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Table 1. Characteristics of the study population

	Cases, *n* (%)	Controls, *n* (%)
Total	1,578	1,966
TCF7L2		
CC	777 (49.2)	1,027 (52.2)
CT	677 (42.9)	786 (40.0)
TT	124 (7.9)	153 (7.8)
Age at selection (y)		
30-39	24 (1.5)	41 (2.1)
40-49	100 (6.3)	127 (6.5)
50-59	292 (18.5)	331 (16.8)
60-69	551 (34.9)	678 (34.5)
70-79	611 (38.7)	789 (40.1)
Center		
KPMCP	762 (48.3)	799 (40.6)
Minnesota	565 (35.8)	798 (40.6)
Utah	251 (15.9)	369 (18.8)
Sex		
Male	884 (56.0)	1,054 (53.6)
Female	694 (44.0)	912 (46.4)
Race		
White, non-Hispanic	1,443 (91.6)	1,834 (93.4)
Hispanic	61 (3.9)	75 (3.8)
African-American	71 (4.5)	55 (2.8)
Recent aspirin/NSAID use		
Yes	490 (33.3)	808 (44.1)
No	982 (66.7)	1,025 (55.9)
Disease stage		
Local	571 (36.2)	
Regional	798 (50.6)	
Distant	135 (8.6)	
Unknown	74 (4.7)	
Tumor site		
Proximal	718 (45.5)	
Distal	731 (46.3)	
Unknown	129 (8.2)	

Abbreviation: KPMCP, Kaiser Permanente Medical Care Program.

body mass index (BMI) and variants of insulin-related genes. We also evaluate aspirin and nonsteroidal anti-inflammatory drug (NSAID) use because high doses of salicylates have been shown to reverse hyperglycemia, hyperinsulinemia, and dyslipidemia by improving sensitivity to insulin signaling (9) and may therefore modify risk associated with the *TCF7L2* polymorphism.

Materials and Methods

Data for the study come from a colon cancer case-control study 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 time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of colorectal cancer, 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 were randomly selected from lists provided by the Centers for Medicare and Medicaid Services (formerly Health Care Financing Administration) and controls <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 (10, 11). The current analysis is restricted to subjects who provided a blood sample from which the *TCF7L2* polymorphism could be genotyped. The colon cancer study population consists of non-Hispanic white cases (n = 1443) and controls (n = 1834), Hispanic cases (n = 61) and controls (n = 75), and African-American cases (n = 71) and controls (n = 55). Two cases and three controls did not report race.

Trained and certified interviewers collected diet and lifestyle data as previously outlined (12, 13). The referent year for the study was the calendar year ~2 y before 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, and body size; and other lifestyle factors, including medical, family, and reproductive history.

DNA was extracted from blood drawn from study participants. Genotyping of the rs7903146 polymorphism was done using a Taqman assay obtained from Applied Biosystems. Briefly, 20 ng of genomic DNA were assessed in each participant using a reaction containing assay-specific probes and primers and 1× Taqman universal PCR master mix (contains AmpErase UNG, AmpliTaq Gold polymerase, and reaction buffer) in a 5 μL final volume. Control samples representing all possible genotypes were included at four positions in every 384-well tray. Internal replicates representing >1% of the sample set were blinded and included.

We assessed interaction between the rs7903146 *TCF7L2* polymorphism and polymorphisms in the *insulin-like growth factor-1* gene (CA repeat and rs5742612), *insulin-like growth factor–binding protein 3* (rs2854744 and rs2854746), *insulin receptor substrate 1* (rs1801278), and *insulin receptor substrate 2* (rs1805097) as previously described (14).

Information on tumor characteristics, including disease stage and survival data, was obtained through local cancer registries. Utah and California tumor registries are part of the national Surveillance, Epidemiology, and End Results program, and the Minnesota registry is a member of the Centers for Disease Control and Prevention–funded cancer registry programs. Additionally, we evaluated microsatellite instability (MSI) in tumors as described previously (15).

Statistical Methods. Statistical Analysis System statistical package version 9.1 (SAS Institute) was used to conduct the analyses. We evaluated the distribution of the genotypes by race and compared the *TCF7L2* polymorphism to the independent associations of genetic polymorphisms with colon cancer. Multivariate logistic regression models were used to evaluate the associations between colon cancer and *TCF7L2* genotypes; multinomial logistic regression models were used to evaluate the associations of tumor characteristics such as MSI and *TCF7L2* genotypes. All logistic regression models were adjusted for age at selection or diagnosis, study center, race or ethnicity, sex, BMI (kg/m^2), and physical activity. Odds ratios (OR) and 95% confidence intervals (95% CI) are used to report associations obtained from the multivariate logistic regression models. Trend is assessed by comparing the log likelihood of a logistic regression model with the variable of interest, entered as an ordered categorical variable, to the log likelihood of a model without the variable of interest using a χ^2 test with one

Table 2. Association between *TCF7L2* rs7903146 polymorphisms and colon cancer

	Controls	Cases	
	n	*n*	OR* (95% CI)
TCF7L2			
CC	1,024	776	1.00
CT	785	673	1.14 (0.99-1.31)
TT	153	124	1.03 (0.80-1.33)
P trend			0.22
TCF7L2			
CC	1,024	776	1.00
CT and TT	938	797	1.12 (0.98-1.28)

*OR and 95% CI adjusted for age, sex, center, race, BMI, and physical activity.

degree of freedom. Multivariate logistic regression models were used to evaluate the joint association of outcome with the *TCF7L2* polymorphism and aspirin/NSAIDs, BMI, physical activity, *insulin receptor substrate 1, insulin receptor substrate 2, insulin-like growth factor-1*, and *insulin-like growth factor–binding protein 3*. Effect modification between genotypes and exposure variables was evaluated by a likelihood ratio test for a multiplicative interaction term in the logistic regression model.

Results

The study population was primarily male, >65 years of age, and non-Hispanic white (Table 1). The *TCF7L2* polymorphism was in Hardy-Weinberg equilibrium in the study population, with a minor allele frequency of 0.28 in non-Hispanic white, 0.29 in African-American, and 0.23 in Hispanic controls.

We observed no statistically significant association between the *TCF7L2* genotypes and risk of colon cancer for all centers combined (Table 2). Center-specific associations showed a statistically significant association for Utah only (OR, 1.48; 95% CI, 1.05-2.03) when using a dominant model that evaluated any T allele relative to the CC genotype. Associations for both the Kaiser Permanente Medical Care Program and Minnesota sites were close to 1.00 and not statistically significant. No sex- or age-specific associations were detected (data not shown in table). Associations with the rs7903146 polymorphism did not differ by proximal or distal colon subsite (data not shown in table). Similarly, null associations were observed for less advanced disease stage (OR for local, 1.08; 95% CI, 0.89-1.30; OR for regional, 1.19; 95% CI, 1.00-1.40; OR for distant disease stage, 1.02; 95% CI, 0.72-1.46). We observed no statistically significant associations between length of survival and the *TCF7L2* polymorphism (data not shown in table).

A statistically significant interaction (P = 0.0001) was observed between recent use of aspirin and or NSAIDs and the *TCF7L2* rs7903146 polymorphism. An inverse association with the T allele was observed among those who reported recent use of aspirin/NSAIDs, whereas an increased risk of colon cancer was observed among those who reported no recent aspirin/NSAID use (Table 3). BMI, physical activity, smoking, and insulin-related genotypes did not interact with the rs7903146 *TCF7L2* marker (data not shown in table).

The *TCF7L2* polymorphism was not associated uniquely with MSI; having either the CT or TT genotypes was associated with a slightly greater influence among microsatellite stable (MSS; OR, 1.16; 95% CI, 0.99-1.36) versus MSI unstable tumors (OR, 1.04; 95% CI, 0.77-1.42). The interaction between NSAIDs and the *TCF7L2* genotype was slightly stronger for MSS versus MSI tumors (Table 4). Among those with a T allele, aspirin modifies the risk of both MSS and MSI tumors. However, the likelihood of a MSI but not MSS tumor is influenced by those carrying the CC genotype and taking aspirin/NSAIDs.

Discussion

The *TCF7L2* rs7903146 polymorphism was not associated with colon cancer overall in this population. However, among those who did not report recent use of aspirin/NSAIDs, we observed a modest significant increased risk of colon cancer. On the other hand, individuals who reported recently using aspirin/NSAIDs were at a reduced risk of colon cancer if they had the T allele of the polymorphism. It is not clear if the underlying proportion of the population that uses aspirin/NSAIDs differs in this study from previous studies; however, our results suggest that use of aspirin/NSAIDs may influence the direction and magnitude of the association between the rs7903146 *TCF7L2* polymorphism and colon cancer.

Aspirin and use of NSAIDs have been shown to influence colon cancer risk in several disease pathways,

Table 3. Association between *TCF7L2* rs7903146 polymorphism and colon cancer by recent use of aspirin/NSAIDS

	Recent aspirin/NSAID use			No recent aspirin/NSAID use			*P* interaction
	Controls	Cases		Controls	Cases		
	n	*n*	OR* (95% CI)	*n*	*n*	OR (95% CI)	
TCF7L2							
CC	394	269	1.00	558	452	1.19 (0.98-1.46)	0.0001
CT	339	186	0.80 (0.63-1.02)	397	449	1.65 (1.35-2.03)	
TT	75	35	0.68 (0.44-1.05)	70	81	1.64 (1.14-2.34)	
P trend			0.03			0.001	
TCF7L2							
CC	394	269	1.00	558	452	1.19 (0.98-1.46)	
CT and TT	414	221	0.78 (0.62-0.98)	467	530	1.65 (1.35-2.02)	

*OR and 95% CI adjusted for age, center, race, sex, BMI, and physical activity.

Table 4. Association between *TCF7L2* genotype and MSS and MSI colon tumors by use of aspirin/NSAIDs

	TCF7L2					
	CC			CT and TT		
	Controls	Cases		Controls	Cases	
	n	*n*	OR* (95% CI)	*n*	*n*	OR (95% CI)
MSS cases vs controls						
Recent aspirin/NSAID use						
Yes	394	180	1.00	414	146	0.77 (0.59-1.00)
No	558	275	1.09 (0.87-1.37)	467	331	1.54 (1.23-1.93)
P interaction		0.0002				
MSI cases vs controls						
Recent aspirin/NSAID use						
Yes	394	30	1.00	414	25	0.81 (0.47-1.39)
No	558	58	1.41 (0.89-2.23)	467	61	1.75 (1.10-2.77)
P interaction		0.07				

*OR and 95% CI adjusted for age, center, race, sex, BMI, and physical activity.

including an insulin-related pathway (16, 17) as well as generally reducing the risk of colon cancer (18, 19). High doses of salicylates have been shown to reverse hyperglycemia, hyperinsulinemia, and dyslipidemia in obese rodents by improving sensitivity to insulin signaling (9). In patients with type 2 diabetes, aspirin treatment has been shown to reduce fasting plasma glucose, total cholesterol, C-reactive protein, triglycerides, and insulin clearance; aspirin reduced hepatic glucose production and improved insulin-stimulated peripheral glucose uptake by 20% (20-25). Insulin resistance and diabetes have been hypothesized to be associated with colon cancer (26), and thus, the *TCF7L2* polymorphism, which also has been associated with diabetes and insulin sensitivity, plausibly may modulate colon cancer risk. Our findings that aspirin/NSAIDs modulate the association between *TCF7L2* and colon cancer are consistent with previous reports on the association between salicylates and insulin. Our data suggest that the influence of the T allele is modified by the use of aspirin and only increases risk in the absence of aspirin/NSAIDs. Our data further suggest that when carrying the T allele aspirin modifies the risk of both MSS and MSI tumors, whereas when carrying the CC genotype aspirin only affects the risk of having a MSI tumor. We did not observe significant interaction between other insulin-related factors analyzed. Although our findings with aspirin/NSAIDs could be from chance because of several comparisons made, all associations evaluated were hypothesized a *priori*.

Unlike the study of Folsom and colleagues, we did not observe a statistically significant association between the T allele of the *TCF7L2* polymorphism overall, although an increased risk was observed in the Utah population. Methodologic differences between the two studies exist, in that our study was a population-based case-control study, and the study previously reported by Folsom[5] was based on prospective follow-up of the Atherosclerosis Risk in Communities cohort. Differences in association could exist if the genotype was associated with survival and those most critically ill did not participate in the case-control study. However, we did not observe any differences in survival based on *TCF7L2* genotype, suggesting that differences in detected association are not the result of selection bias based on poorer response among those who are most ill. The reasons for these differences may therefore be the result of differences in other characteristics of the population, such as recent use of aspirin/NSAIDs, or unmeasured covariates.

The rs7903146 *TCF7L2* polymorphism has been associated with development of type 2 diabetes and with insulin sensitivity and secretion (1, 2, 27, 28). Additionally, *TCF7L2* is involved in the Wnt/β-catenin pathway, which is critical to proper functioning of the *APC* gene involved in colon cancer carcinogenesis (4, 5). Although it is a plausible candidate gene that may be associated with colon cancer, we observed a statistically significant increased risk of colon cancer only among those not using aspirin/NSAIDs; further, in the presence of homozygous C alleles, taking aspirin seems to modify risk of MSI^+ tumors rather than MSS tumors, whereas for those with one or two T alleles aspirin seems to modify risk of both MSS and MSI tumors. These results need to be confirmed in other studies.

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