Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2008 April ; 17(4): 978–982. doi:10.1158/1055-9965.EPI-07-2687.

TCF7L2 polymorphism and colon cancer

Martha L. Slattery[1], **Aaron R. Folsom**[2], **Roger Wolff**[1], **Jenifer Herrick**[1], **Bette J. Caan**[2], and **John D. Potter**[4]

1 *Department of Internal Medicine, University of Utah School of Medicine, Salt Lake City, Utah*

2 *Division of Epidemiology and Community Health, University of Minnesota School of Public Health, Minneapolis, Minnesota*

3 *Kaiser Permanente Medical Research Program, Oakland, California*

4 *Fred Hutchinson Cancer Research Center, Seattle, Washington*

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 1578 cases and 1966 controls. We did not observe a statistically significant association between the rs7903146 polymorphisms and risk of colon cancer (OR 1.12 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/NSAIDs (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 two years prior to 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 colon cancer risk associated with the rs7903146 *TCF7L2* polymorphism is modified by use of aspirin/NSAIDs.

Keywords

colon cancer; TCF7L2; aspirin; non-steroidal anti-inflammatory drugs

---

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* has been shown to be involved in the *Wnt*/β-catenin signaling pathway (3) which is known to be 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 (ARIC) Study suggest that an association exist (Folsom under review). In their study, the TT genotype of the rs7903146 *TCF7L2* gene was associated with a greater than twofold increased risk of colon cancer (HRR 2.15 95% CI 1.27,3.64).

Using data from a large multi-center 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 associated with insulin-related factors. Factors evaluated include BMI and variants of insulin-related genes. We also evaluate aspirin and non-steroidal anti-inflammatory drug use since 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.

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 years 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 five-year age groups. At the KPMCP, controls were randomly selected from membership lists. In Utah, controls 65 years and older were randomly selected from lists provided by the Centers for Medicare and Medicaid Services (formerly HCFA) 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 (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 approximately two years prior to 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 non-steroidal drug use, 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 performed using a TaqMan assay obtained from Applied Biosystems (Foster City, CA). Briefly, 20 ng of genomic DNA was assessed in each participant using a reaction containing assay specific probes and primers, 1X TaqMan universal PCR master mix (contains AmpErase UNG, AmpliTaq Gold polymerase, and reaction buffer) in a 5μl 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 insullin-like growth factor-1 (*IGF1*) gene (CA repeat and rs5742612), insulin-like growth factor binding protein-3 (*IGFBP3*) (rs2854744 and rs2854746), insulin-receptor substrate 1 (*IRS1*) (rs1801278) and 2 (I*RS2*) (rs1805097) as previously described (14).

Information on tumor characteristics including disease stage and survival data were obtained through local cancer registries. Utah and California tumor registries are part of the national

Surveillance, Epidemiology, and End Results program (SEER), and the Minnesota registry is a member of the CDC funded cancer registry programs. Additionally, we evaluated microsatellite instability in tumors as described previously (15).

Statistical Methods

SAS statistical package, version 9.1 (SAS Institute Cary, NC) 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/m2), and physical activity Odds ratios (OR) and 95% confidence intervals (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 chi-square test with one 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, *IRS1*, *IRS2*, *IGF1*, and *IGFBP3*. Effect modification between genotypes and exposure variables were evaluated by a likelihood ratio test for a multiplicative interaction term in the logistic regression model.

Results

The study population was primarily male, older than 65 years of age, and non-Hispanic white (NHW) (Table 1). The *TCF7L2* polymorphism was in Hardy Weinberg Equilibrium in the study population, with a minor allele frequency of 0.28 in NHW, 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 KPMCP 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 sub-site (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 an 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 a number of disease pathways, 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 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. While our findings with aspirin/NSAIDs could be from chance because of several comparison made, all associations evaluated were hypothesized a prior.

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. Methodological differences between the two studies exist, in that our study was a population-based case-control study, and the study previously reported by Folsom (under review) was based on prospective follow-up of the ARIC 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 known to be 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 appears to modify risk of MSI+ tumors rather than MSS tumors, whereas for those with one or two T alleles, aspirin appears to modify risk of both MSS and MSI tumors. These results need to be confirmed in other studies.

Acknowledgements

This study was funded by NCI grants CA48998, CA85846, and CA61757. This research also was supported by the Utah Cancer Registry, which is funded by Contract #N01-PC-67000 from the National Cancer Institute, with additional support from the State of Utah Department of Health, the Northern California Cancer Registry, and the Sacramento Tumor Registry. 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. We would like to acknowledge the contributions of Michael Hoffman and Thao Tran for genotyping and Sandra Edwards, Karen Curtin, Roger Edwards, Leslie Palmer, Donna Schaffer, Dr. Kristin Anderson, and Judy Morse for data management and collection.

References

1. Salonen JT, Uimari P, Aalto JM, Pirskanen M, Kaikkonen J, Todorova B, et al. Type 2 diabetes whole-genome association study in four populations: the DiaGen consortium. Am J Hum Genet 2007;81:338–45. [PubMed: 17668382]
2. Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, et al. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest 2007;117:2155–63. [PubMed: 17671651]
3. Prunier C, Hocevar BA, Howe PH. Wnt signaling: physiology and pathology. Growth Factors 2004;22:141–50. [PubMed: 15518237]
4. Wong NA, Pignatelli M. Beta-catenin--a linchpin in colorectal carcinogenesis? Am J Pathol 2002;160:389–401. [PubMed: 11839557]
5. Samowitz WS, Slattery ML, Sweeney C, Herrick J, Wolff RK, Albertsen H. APC mutations and other genetic and epigenetic changes in colon cancer. Mol Cancer Res 2007;5:165–70. [PubMed: 17293392]
6. Domenyuk VP, Litovkin KV, Verbitskaya TG, Dubinina VG, Bubnov VV. Identification of new DNA markers of endometrial cancer in patients from the ukrainian population. Exp Oncol 2007;29:85–93. [PubMed: 17704738]
7. Burwinkel B, Shanmugam KS, Hemminki K, Meindl A, Schmutzler RK, Sutter C, et al. Transcription factor 7-like 2 (TCF7L2) variant is associated with familial breast cancer risk: a case-control study. BMC Cancer 2006;6:268. [PubMed: 17109766]
8. Schneikert J, Behrens J. The canonical Wnt signalling pathway and its APC partner in colon cancer development. Gut 2007;56:417–25. [PubMed: 16840506]
9. Yuan M, Konstantopoulos N, Lee J, Hansen L, Li ZW, Karin M, et al. Reversal of obesity- and diet-induced insulin resistance with salicylates or targeted disruption of Ikkbeta. Science 2001;293:1673–7. [PubMed: 11533494]
10. Slattery ML, Edwards S, Curtin K, Ma K, Edwards R, Holubkov R, et al. Physical activity and colorectal cancer. Am J Epidemiol 2003;158:214–24. [PubMed: 12882943]
11. Slattery ML, Potter J, Caan B, Edwards S, Coates A, Ma KN, et al. Energy balance and colon cancer--beyond physical activity. Cancer Res 1997;57:75–80. [PubMed: 8988044]
12. Edwards S, Slattery ML, Mori M, Berry TD, Caan BJ, Palmer P, et al. Objective system for interviewer performance evaluation for use in epidemiologic studies. Am J Epidemiol 1994;140:1020–8. [PubMed: 7985650]
13. Slattery ML, Caan BJ, Duncan D, Berry TD, Coates A, Kerber R. A computerized diet history questionnaire for epidemiologic studies. J Am Diet Assoc 1994;94:761–6. [PubMed: 8021418]
14. Slattery ML, Samowitz W, Curtin K, Ma KN, Hoffman M, Caan B, et al. Associations among IRS1, IRS2, IGF1, and IGFBP3 genetic polymorphisms and colorectal cancer. Cancer Epidemiol Biomarkers Prev 2004;13:1206–14. [PubMed: 15247132]
15. Slattery ML, Curtin K, Anderson K, Ma KN, Ballard L, Edwards S, et al. Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. J Natl Cancer Inst 2000;92:1831–6. [PubMed: 11078760]

16. Camp NJ, Slattery ML. Classification tree analysis: a statistical tool to investigate risk factor interactions with an example for colon cancer (United States). Cancer Causes Control 2002;13:813–23. [PubMed: 12462546]
17. Slattery ML, Samowitz W, Hoffman M, Ma KN, Levin TR, Neuhausen S. Aspirin, NSAIDs, and colorectal cancer: possible involvement in an insulin-related pathway. Cancer Epidemiol Biomarkers Prev 2004;13:538–45. [PubMed: 15066917]
18. Marnett LJ. Aspirin and the potential role of prostaglandins in colon cancer. Cancer Research 1992;52:5575–5589. [PubMed: 1394181]
19. Sandler RS. Aspirin and other nonsteroidal anti-inflammatory agents in the prevention of colorectal cancer. Important Adv Oncol 1996:123–37. [PubMed: 8791132]
20. Hundal RS, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, et al. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. J Clin Invest 2002;109:1321–6. [PubMed: 12021247]
21. Fang V, Foye WO, Robinson SM, Jenkins HJ. Hypoglycemic activity and chemical structure of the salicylates. J Pharm Sci 1968;57:2111–6. [PubMed: 5708353]
22. Graef I, Gibbons DM. Salicylates and carbohydrate metabolism. Diabetes 1960;9:416–8. [PubMed: 13707654]
23. Powell ED, Field RA. Studies on salicylates and complement in diabetes. Diabetes 1966;15:730–3. [PubMed: 5924835]
24. McRae JR, Chen M, Robertson RP. Improvement of defective insulin responses to glucose, arginine, and beta-adrenergic stimulation in diabetics by sodium salicylate. Adv Prostaglandin Thromboxane Res 1980;8:1287–9. [PubMed: 6769309]
25. Baron SH. Salicylates as hypoglycemic agents. Diabetes Care 1982;5:64–71. [PubMed: 6754304]
26. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. J Nutr 2001;131:3109S–20S. [PubMed: 11694656]
27. Scott LJ, Mohlke KL, Bonnycastle LL, Willer CJ, Li Y, Duren WL, et al. A genome-wide association study of type 2 diabetes in Finns detects multiple susceptibility variants. Science 2007;316:1341–5. [PubMed: 17463248]
28. Wang J, Kuusisto J, Vanttinen M, Kuulasmaa T, Lindstrom J, Tuomilehto J, et al. Variants of transcription factor 7-like 2 (TCF7L2) gene predict conversion to type 2 diabetes in the Finnish Diabetes Prevention Study and are associated with impaired glucose regulation and impaired insulin secretion. Diabetologia 2007;50:1192–200. [PubMed: 17437080]

Table 1
Characteristics of the study population

	Cases		Controls	
	n	%	n	%
Total	**1578**		**1966**	
TCF7L2				
CC	777	49.2	1027	52.2
CT	677	42.9	786	40.0
TT	124	7.9	153	7.8
Age at selection				
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	1054	53.6
Female	694	44.0	912	46.4
Race				
White, non-Hispanic	1443	91.6	1834	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	1025	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		

Table 2

Association between *TCF7L2* rs7903146 polymorphisms and colon cancer

	Controls	Cases		
	n	n	OR[1]	(95% CI)
TCF7L2				
CC	1024	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	1024	776	1.00	
CT and TT	938	797	1.12	(0.98, 1.28)

[1]Odds ratios and 95% Confidence Intervals (CI) adjusted for age, sex, center, race, BMI, and physical activity

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				
	Controls	Cases			Controls	Cases			
	n	n	OR[1]	(95% CI)	n	n	OR	(95% CI)	p interaction
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)	

[1]Odds ratio (OR) and 95% confidence Intervals (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					

[1] Odds ratio (OR) and 95% confidence Intervals (CI) adjusted for age, center, race, sex, BMI, and physical activity.