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Toll-like receptor genes and their association with colon and rectal cancer development and prognosis

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Abstract

Toll-like receptors (TLR) are mediators of inflammation in the gut and potentially important modulators of colon and rectal cancer risk.

We use data from a population-based study of incident colon cancer cases (n=1,555) and controls (n=1,956) and rectal cancer cases (n=754) and controls (n=959). We evaluate genetic variation in *TLR2* (6 SNPs), *TLR3* (4 SNPs), and *TLR4* (8 SNPs) with risk of developing colon or rectal cancer and survival after diagnosis.

TLR3 rs11721827 was associated with rectal cancer (OR 1.27 95% CI 1.02,1.58 for AC/CC vs AA genotype Wald p 0.035; adjusted p 0.126); *TLR3* rs3775292 and *TLR4* rs11536898 were associated with colon cancer (OR 0.68 95% CI 0.49,0.95 for GG vs CC/CG and OR 0.50 95% CI 0.29,0.87 for AA vs. CA/CC respectively; Wald p=0.023 and 0.015; adjusted p=0.085 and 0.101 respectively). *TLR2* rs7656411 and rs3804099 respectively interacted with NSAID use and cigarette smoking to alter risk of colon cancer (adjusted p=0.034 and 0.077); *TLR3* rs11721827 interacted with NSAID use to alter risk of colon cancer (adjusted p=0.071). *TLR3* rs3775292 interacted with dietary carbohydrates to alter colon cancer risk and with dietary carbohydrates and saturated fat to alter rectal cancer risk (adjusted p=0.064, 0.0035, and 0.025 respectively). Multiple SNPs in *TLR2* and *TLR4* were associated with colon cancer survival. Although few independent associations with *TLR* genes were observed, we observed significant interaction with *TLR2* and *TLR3* with hypothesized lifestyle factors. Interaction with dietary factors remained significant for rectal cancer after adjustment for multiple comparisons.

Keywords

Colon Cancer; Rectal Cancer; Toll-like receptors (TLR); NSAID; cigarette smoking; diet; survival

Introduction

The role of inflammation in the development of colon and rectal cancer is well documented [1]. Toll-like receptors (TLR) are mediators of inflammation in the gut and may therefore be important modulators of colon and rectal cancer risk [2]. TLR are crucial regulators that help maintain the balance between commensal bacteria in the gut and the mucosal immune system [3]. Breakdown of the homeostasis in the gut may be a key feature in the

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pathogenesis of inflammatory bowel disease and possibly of risk of colon and rectal cancer. Several TLRs have been examined in the intestine. TLR3 is expressed in normal intestinal epithelial cells [2]; TLR4 is up-regulated in Crohn's disease and ulcerative colitis; expression of TLR4 and TLR2 is increased in lamina propria macrophages in inflammatory bowel disease [4, 5]. TLR4 also induces COX2 and is important for proliferation and apoptosis in response to intestinal injury. A study by Xiao and colleagues [6] showed that decreased TLR signaling resulted in increased intestinal inflammation further suggesting the importance of TLR signaling in colon carcinogenesis [2]. Studies also have demonstrated that TLRs play a role in progression of polyps to tumors and that reduced TLR4 expression is associated with increased metastatic potential of colorectal cancers [7, 8]. TLR5 has been shown to protect the gut from enteric microbes [9].

The effects of genetic variation in the *TLR* genes have been examined in a few studies of Crohn's disease and cancer. A *TLR2* GT repeat microsatellite and *TLR4* D299G were associated with colorectal cancer in a study of 89 cancer cases [10]. *TLR2* and *TLR4* were examined in 182 Crohn's disease patients [11]. The *TLR4* D299G polymorphism was significantly associated with increased risk of Crohn's disease. SNP rs3775291 of the *TLR3* gene was associated with colorectal cancer (CRC) survival when examined among 614 CRC patients in Germany [12]. The impact was greatest among those diagnosed with stage II cancers.

While there are suggestions of the importance of TLRs in colon and rectal cancer etiology and survival, there is limited information on associations with genetic variation in these genes. It is reasonable to hypothesize that exposure to aspirin/NSAIDs, dietary fat and carbohydrates, and cigarette smoking may modify the risk associated with TLR genes. TLR4 has been shown to be required for inducing COX2 expression following intestinal epithelial cell injury [3]. Commensal bacteria which are essential for maintenance of homeostasis the gut have been shown to be influenced by dietary fat, especially saturated fats, and carbohydrates [13] and TLRs are regulators of this homeostasis. Oxidative stress may enhance intestinal injury and inflammation; cigarette smoking has been shown to influence oxidative stress [14]. In this study we examine colon and rectal cancer risk associated with genetic variation in *TLR2, TLR3*, and *TLR4*; we evaluate if NSAID use, dietary intake of fat and carbohydrates, and cigarette smoking interact with these genes to alter risk; we assess the association between genetic variation in these TLR genes and survival.

Methods

Two study populations are included. The first, a population-based case-control study of colon cancer, included cases (n=1,555) and controls (n=1,956) identified between October 1, 1991 and September 30, 1994 living in the Twin Cities Metropolitan Area, Kaiser Permanente Medical Care Program of Northern California (KPMCP) and a seven-county area of Utah [15]. The second study used identical data collection methods as the first study but included population-based cases with cancer of the rectosigmoid junction or rectum (n=754) and controls (n=959) who were identified between May 1997 and May 2001 in Utah and KPMCP [16]. Eligible cases were between 30 and 79 years old at 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 matched to cases by sex 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

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driver's license lists. While in Minnesota, controls were selected from driver's license and state-identification lists. Study details have been previously reported [15, 16].

Interview Data Collection

Data were collected by trained and certified interviewers using laptop computers. All interviews were audio-taped and reviewed for quality control purposes [17]. The referent period for the study was two years prior to diagnosis for cases and prior to selection for controls. Detailed information was collected on diet [18] physical activity, medical history, cigarette smoking history, regular use of aspirin and non-steroidal anti-inflammatory drugs, and body size. The NCC nutrient database was used to calculate nutrients from reported foods on the extensive diet history questionnaire.

Tumor Registry Data

Tumor registry data were obtained to determine disease stage at diagnosis and months of survival after diagnosis. Disease stage was categorized using the sixth edition of the American Joint Committee on Cancer (AJCC) staging criteria. Disease staging was done centrally by one pathologist in Utah. Local tumor registries also provided information on patient follow-up including vital status, cause of death, and contributing cause of death. Follow-up was obtained for all study participants and was terminated for the Colon Cancer Study in 2000 and for the Rectal Cancer Study in 2007. At that time all study participants had over five years of follow-up.

TagSNP Selection and Genotyping

All SNPs evaluated were tagSNPs and selected using the following parameters: LD blocks were defined using a Caucasian LD map and an r^2=0.8; minor allele frequency (MAF) >0.1; range= -1500 bps from the initiation codon to +1500 bps 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, San Diego, California). 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%. Individuals with missing genotype data were not included in the analysis for that specific marker. A description of the genes and corresponding SNPs is available in supplement table 1; supplement table 2 details the distribution of the genotypes for cases and controls for all SNPs assessed. All SNPs were in Hardy Weinberg Equilibrium.

Statistical Methods

Statistical analyses were performed using SAS® version 9.2 (SAS Institute, Cary, NC). We report odds ratios (ORs) and 95% confidence intervals (95%CIs) assessed from adjusted multiple logistic regression models adjusting for age, center, race/ethnicity (approximately 90% Caucasian, 5% African American and 5% Hispanic), and sex which were matching variables for the original study. Inheritance models were selected based on initial review of results from the additive model. When risk estimates indicated that a recessive or dominant model was more appropriate that model was used. If the minor allele frequency was too low (less than 0.1 for evaluation of main effects; less than 0.15 for evaluation of interactions) then a dominant model was assigned. Analysis for interaction was based on tagSNPs within each *TLR* gene, unadjusted for other SNPs. Diet and lifestyle variables were selected because of their biological plausibility for involvement in this candidate pathway. Interactions between *TLR* genes and hypothesized exposures associated with inflammation and oxidative stress (i.e. recent aspirin or NSAID use) and cigarette smoking (recent or not recent smoker), or their effect on commensal bacteria in the gut (saturated fat and carbohydrates). Aspirin/NSAID use and cigarette smoking were categorized into two levels

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to maximize power. Nutrients were determined from the University of Minnesota Nutrition Data System Nutrient Database that was used to covert reported foods into nutrients. Tertiles of dietary intake per 1000 calories were based on the sex-specific distribution in controls. For colon cancer the cut points for carbohydrates were 260g and 366g for men and 205g and 282g for women; for saturated fat the cut points were 23.8g and 38.6g for men and 17.7g and 28.1g for women. The corresponding cut points for the rectal cancer study for carbohydrate were 271g and 290g for men and 220g and 318g for women and for saturated fat were 24.4g and 41.7g for men and 20.6g and 32.6g for women. *P* values for interaction were determined using a likelihood-ratio test comparing a full model that included an interaction term with a reduced model without an interaction term.

Survival-months were calculated based on month and year of diagnosis and month and year of death or date of last contact. Associations between SNPs and risk of dying of colorectal cancer within five years after diagnosis were evaluated using Cox proportional hazards models to obtain multivariate hazard rate ratios (HRRs) and 95% confidence intervals. We adjusted for age at diagnosis, study center, race, sex, tumor molecular phenotype, and AJCC stage to estimate HRRs.

Adjusted multiple-comparison p values (pACT), taking into account tagSNPs within the gene, were estimated using the methods by Conneely and Boehnke [19] via R version 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria). Wald p values from the main effect models and interaction p values based on likelihood-ratio tests were used for estimates of multiple comparisons. We consider a pACT of <0.20 as being potentially important given the candidate pathway approach and the need to consider both type 1 and type 2 errors. We believe that findings at this level would merit replication.

Results

We detected few statistically significant associations between *TLR2, TLR3*, and *TLR4* and colon and rectal cancer (Table 1). The only main effect association that was significant for colon cancer was with *TLR4*, where the AA genotype of rs11536898 was inversely associated with colon cancer risk (OR 0.50, 95% CI 0.29, 0.87). After adjustment for multiple comparisons, the pACT was 0.101 compared to the Wald p value from the unadjusted model of 0.0148. *TLR3* rs11721827, was significantly associated with rectal cancer (OR 1.27 95 % CI 1.02,1.58); the adjusted pACT value was 0.127 while the unadjusted values was 0.0351.

Evaluation of interaction between diet and lifestyle factors showed significant interaction for both *TLR2* and *TLR3* and NSAID use and for *TLR2* and cigarette smoking for colon cancer (Table 2). Lower risk was associated with having recently used aspirin/NSAIDsin the presence of the variant allele of *TRL2* rs7656411 and *TLR3* rs11721827; genotype did not influence risk among those who did not recently use aspirin/NSAID. Having the CC genotype of *TLR2* rs3804099 reduced risk associated with cigarette smoking while having no impact among non-smokers. *TLR3* rs3775292 interacted with both total carbohydrates for colon and rectal cancer and with saturated fat for rectal cancer. While there was significant interaction for both colon and rectal cancer with this polymorphism, the direction of the association was different. The CG/GG genotypes were associated with reduced risk of rectal cancer at high levels of carbohydrate intake and slight non-significant increased risk at low levels of intake, while the opposite was true for colon cancer. Similar patterns of association were observed for saturated fat intake and rectal cancer.

Both *TLR2* and *TLR4* were associated with survival after diagnosis with colon cancer, but not with rectal cancer (Table 3). *TLR2* rs5743704 and rs5743708 were associated with poorer survival (HRRs 1.89 95% CI 1.26, 2.83; and 1.74 95% CI 1.12, 2.70 respectively).

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These two SNPs were not in LD, with R^2 values of <0.01. Having the CC genotype of rs1554973 of *TLR4* was associated with better survival (HRR 0.55 95% CI 0.31,0.98) after diagnosis with colon cancer. Stratification by disease stage at time of diagnosis into those with AJCC stages 1 and 2 and those with stage III or IV, showed two significant associations (data not shown in table). *TLR2* rs5743704 CA/AA genotypes were associated with worse survival when diagnosed with stage III or IV (HRR 2.10 95% CI 1.23,3.59), while there was no impact on survival among those diagnosed at stages I or II. *TLR3* rs3775292 CG/GG genotypes were associated with slightly better survival if diagnosed at more advanced disease stage (HRR 0.74 95% CI 0.56,0.99), while there was no impact on survival among those with disease stages I or II at diagnosis (HRR 0.96 95% CI 0.55,1.67). There were no significant associations with survival after diagnosis with rectal cancer for any of the TLR genotypes.

Discussion

Few associations were observed between TLR genes and risk of developing colon or rectal cancer. Associations were restricted primarily to colon cancer, although one SNP in *TLR3* showed a modest unadjusted significant association with rectal cancer. The rs11536898 of *TLR4* was associated with reduced risk of colon cancer; *TLR2* rs7656411 and *TLR3* rs11721827 interacted with NSAIDs use while *TLR2* rs3804099 interacted significantly with cigarette smoking. *TLR3* rs3775292 interacted with carbohydrate and saturated fat intake. Both *TLR2* and *TLR4* polymorphisms were associated with survival after diagnosis with colon cancer.

Both NSAID use and cigarette smoking interacted with genetic variation in *TLR2* and *TLR3* as hypothesized. While most studies have focused on TLR4 and have shown that is required for the induction of COX2 in response to injury [3], TLR2 and TLR3 also have been shown to have a protective effect against intestinal injury. Given that NSAIDs are a COX2 inhibitor, it is possible that the influence of TLRs that stem from genetic variation in these genes may be influenced by the presence of COX2 inhibitors such as NSAID use. Smoking cigarettes can influence cellular oxidative stress; studies have further shown that oxidants in cigarette smoke inhibit pathogen recognition receptor function that may involve TLRs [14] TLR2 also has been shown to provide a signal that initiates inflammatory response in mice in a state of oxidative stress [5]. Our observed interaction between cigarette smoking and *TLR2* polymorphisms is biologically plausible; we observed that in the presence of cigarette smoke, variation in the *TLR2* gene influenced risk of colon cancer.

Dietary components may have an important role in regulating gut microflora. Intake of a high fat, especially high saturated fat, and high carbohydrate diet that influence gut microflora can induce inflammation, oxidative stress, and endotoxins as well as TLR expression [20]. Intestinal microflora can stimulate the immune system through TLR and negative regulation of TLRs is essential for maintaining intestinal homeostasis [21]. We observed that *TLR3* rs3775292 significantly interacted with dietary carbohydrates for both colon and rectal cancer, while the same SNP also interacted significantly with saturated fat for rectal cancer. Given the same polymorphism was associated with colon and rectal cancer for both carbohydrates and saturated fat we believe that these observations should be replicated in other studies. It is not clear why this interaction would be seen only for *TLR3* genetic variation. It is possible that since TLR3 is expressed in normal colon tissue it may play an important role at the early stages of tumor initiation. If that were the case, this would imply that dietary factors such as high intake of carbohydrates and saturated fat may function as initiators in the carcinogenic process.

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Studies have shown that TLR4 promotes the development of colitis-associated tumors [4, 22]. Other studies have shown that TLR4 is associated with metastatic status of colorectal cancer [7]. TLR4 expression has been shown in normal colon epithelium and CRC cell lines, while a loss of expression has been shown to increase metastases [7]. Other studies have shown that TLR2 expression increased as stage increased from normal tissue to stage III tumors, while TLR3 expression decreased [8]. While the evidence is somewhat limited, there is support that TLRs may influence survival. *TLR3* rs3775291 of the *TLR3* gene was associated with colorectal cancer (CRC) survival among those diagnosed at stage II in a study of 614 CRC patients in Germany [12]. This finding was not replicated in our patient population. We did observe that *TLR3* rs3775292 CG/GG genotypes were associated with slightly improved survival among those diagnosed with more advanced colon cancer. However, our data support that genetic variation in *TLR2* and *TLR4* influence survival for colon cancer but not rectal cancer. GWAS studies have not identified TLR genes among their top few hits, however of the four independent associations detected, three were for the homozygote rare variant and they may not have the ability to detect these associations. Additionally, GWAS have generally not taken into account the influence of diet and lifestyle factors; our data suggest that interaction with these factors is needed to define the risk associated with genes. These findings need replication in other studies.

The study has many strengths, the data set is rich in exposure data, genetic data, tumor marker data, and survival information. Additionally, we are able to evaluate colon and rectal cancer separately given the vast literature that suggest that these are two separate diseases [16, 23-26]. Because of this, we have the ability to thoroughly evaluate *TLR* to determine not only broad independent associations, but also to help identify molecular pathways through which they may operate. In doing this, we made many comparisons, thus we determined p values that took into account these comparisons. The study is hypothesis driven, examining factors that may influence genetic susceptibility. However, a limitation is lack of information on functionality of polymorphisms examined in this study. Our results provide insight for follow-up studies that evaluate these polymorphisms to determine their functionality. Study limitations include our inability to evaluate rare SNPs that may be associated with colon or rectal cancer. While the SNP selection criteria we used to haplotype the gene provided coverage at those specified levels in the methods, it is possible that we do not completely cover the gene given our lack of assessment of extremely rare variants. Additionally, we were limited to markers that were designable on our selected platform. In developing our custom platform we identified approximately 200 genes along a candidate pathway that we hypothesized were important. Our analysis approach has been to evaluate smaller components of the pathway and thus not adjust for genes that we have not analyzed that are on the platform. We believe that this approach is legitimate and correlates with approaches in epidemiology that do not adjust for every item on a questionnaire when a more focused analysis is being conducted. We believe that avoiding false negatives is important and that replication of potentially important findings is needed by other studies.

In summary, we provide links between *TLR* genes which have been hypothesized as important to the carcinogenic process of colorectal cancer. Our data suggest that genetic variation in *TLR2, TLR3*, and *TLR4* may influence the development of colon cancer as well as influence of survival after diagnosis with colon cancer. As hypothesized, these associations were influenced by recent use of NSAIDs and cigarettes. Dietary carbohydrates and saturated fat appeared to influence risk associated with these genes for both colon and rectal cancer. Replication of these findings is warranted given the biological plausibility of the association and the interaction with diet and lifestyle factors that can modify these genetic effects. Further, we believe that this study provides an important mechanistic link that could be the starting point for further studies and important SNPs in these genes could possibly serve as biomarkers for decision making in colorectal cancer treatment.

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Supplementary Material

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Table 1

Summary of SNPs

Gene	Location	tagSNP	Major/Minor Allele	MAF[1]	Colon: Heterozygote OR (95% CI)[2]	Colon: Homozygote Rare OR (95% CI)	Rectal: Heterozygote OR (95% CI)	Rectal: Homozygote Rare OR (95% CI)
TLR2	4q32	rs1898830	A/G	0.33	1.04 (0.91, 1.20)	0.99 (0.80, 1.24)	1.11 (0.90, 1.36)	1.03 (0.75, 1.41)
		rs4696483	C/T	0.13	1.01 (0.86, 1.18)	0.94 (0.61, 1.44)	1.01 (0.80, 1.27)	0.60 (0.27, 1.36)
		rs3804099	T/C	0.44		**0.85 (0.72, 1.00)**[3]	0.98 (0.78, 1.21)	1.00 (0.76, 1.32)
		rs7656411	T/G	0.25	0.92 (0.80, 1.06)	0.96 (0.73, 1.25)	1.21 (1.00, 1.47)	
		rs5743704	C/A	0.04	1.04 (0.81, 1.32)		1.03 (0.71, 1.49)	
		rs5743708	G/A	0.03	1.24 (0.92, 1.68)		0.73 (0.45, 1.19)	
TLR3	4q35	rs5743305	T/A	0.38	1.05 (0.91, 1.21)	1.05 (0.86, 1.28)	0.86 (0.70, 1.06)	0.87 (0.65, 1.17)
		rs11721827	A/C	0.15	0.94 (0.80, 1.10)	0.78 (0.49, 1.25)	**1.27 (1.02, 1.58)**	
		rs3775292	C/G	0.21		**0.68 (0.49, 0.95)**	0.84 (0.68, 1.02)	
		rs3775291	G/A	0.31	1.03 (0.89, 1.18)	1.04 (0.83, 1.32)		0.73 (0.52, 1.03)
TLR4	9q32-q33	rs10759932	T/C	0.15	0.99 (0.85, 1.16)	0.83 (0.53, 1.30)	0.94 (0.75, 1.17)	1.22 (0.66, 2.27)
		rs1927911	C/T	0.27	0.98 (0.85, 1.13)	1.03 (0.80, 1.33)	0.94 (0.77, 1.15)	0.98 (0.67, 1.42)
		rs5030728	G/A	0.29	1.12 (0.97, 1.29)	1.09 (0.85, 1.39)	1.15 (0.94, 1.41)	0.88 (0.60, 1.28)
		rs11536898	C/A	0.13		**0.50 (0.29, 0.87)**	0.88 (0.70, 1.11)	1.27 (0.55, 2.92)
		rs12377632	T/C	0.38	0.96 (0.83, 1.11)	0.84 (0.68, 1.03)	0.98 (0.80, 1.21)	1.13 (0.84, 1.52)
		rs11536889	G/C	0.15	0.91 (0.77, 1.06)	0.82 (0.49, 1.37)	0.94 (0.76, 1.17)	1.25 (0.64, 2.43)
		rs1554973	T/C	0.25		0.79 (0.61, 1.03)	0.88 (0.72, 1.08)	0.99 (0.68, 1.46)
		rs4986791	C/T	0.06	1.08 (0.87, 1.35)		0.99 (0.73, 1.35)	

[1] Minor Allele Frequency (MAF) based on white control population.

[2] Dominant model listed in 'Heterozygote OR' column where comparison is heterozygote and variant homozygote is compared to wildtype homozygote; recessive model listed in 'Homozygote Rare OR' column compares variant homozygote to other genotypes; additive or co-dominant model is designated by listing risk estimates in both 'Heterozygote OR' and 'Homozygote Rare OR' and both genotypes compared to wildtype homozygote.

[3] Wald p value for *TLR2* rs3804099 was 0.048 and pACT p value was 0.238; for TLR3 rs3775292 the Wald p value was 0.023 and the pACT p value was 0.085; for *TLR4* rs11536898 the Wald p value was 0.015 and pACT p value was 0.101; for TLR3 rs11721827 the Wald p value was 0.035 and the pACT was 0.126.

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Table 2

Interaction between TLR polymorphisms and risk of colon cancer

	Controls N	Cases N	OR[1]	(95% CI)	Controls N	Cases N	OR	(95% CI)	Controls N	Cases N	OR	(95% CI)	P interaction Raw/pACT
	No Recent Aspirin/NSAID Use				Recent Aspirin NSAID Use								
Colon Cancer													
TLR2 (rs7656411)													0.012/0.034
TT	620	561	1.00		425	288	0.75	(0.62, 0.91)					
TG	435	404	1.02	(0.85, 1.22)	322	171	0.58	(0.47, 0.73)					
GG	81	88	1.14	(0.82, 1.58)	57	26	0.47	(0.29, 0.77)					
TLR3 (rs11721827)													0.032/0.071
AA	829	759	1.00		568	373	0.72	(0.61, 0.84)					
AC/CC	307	294	1.05	(0.87, 1.27)	236	112	0.53	(0.42, 0.68)					
	Non Smoker/Non Recent Smoker				Recent Smoker								
Colon Cancer													
TLR2 (rs3804099)													0.026/0.077
TC/CC	1261	979	1.00		269	273	1.27	(1.05, 1.54)					
CC	348	255	0.93	(0.77, 1.11)	77	45	0.71	(0.49, 1.04)					
	Tertile 1				Tertile 2				Tertile 3				
	Carbohydrates												
Colon Cancer													
TLR3 (rs3775292)													0.038/0.064
CC/CG	609	465	1.00		617	501	1.00	(0.83, 1.19)	628	531	0.91	(0.72, 1.16)	
GG	42	16	0.49	(0.27, 0.88)	36	17	0.57	(0.31, 1.03)	24	24	1.05	(0.58, 1.93)	
Rectal Cancer													
TLR3 (rs3775292)													0.0017/0.0035
CC	212	163	1.00		192	149	0.86	(0.63, 1.17)	182	182	0.83	(0.56, 1.22)	
CG/GG	114	103	1.20	(0.86, 1.68)	120	79	0.73	(0.51, 1.05)	139	78	0.46	(0.29, 0.71)	
	Saturated Fat												
Rectal Cancer													
TLR3 (rs3775292)													
CC	207	141	1.00		199	152	1.09	(0.80, 1.49)	180	201	1.52	(1.05, 2.20)	0.014/0.025

	Controls N	Cases N	OR[1]	(95% CI)	Controls N	Cases N	OR	(95% CI)	Controls N	Cases N	OR	(95% CI)	P interaction Raw/pACT
	No Recent Aspirin/NSAID Use				Recent Aspirin NSAID Use								
CG/GG	115	88	1.14	(0.80, 1.63)	122	78	0.92	(0.64, 1.33)	136	94	0.95	(0.62, 1.43)	

[1] Odds Ratio (OR) and 95% Confidence Intervals (CI) adjusted for age, race/ethnicity, sex, and center; dietary factors adjusted for total energy intake

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Table 3

Associations between TLR genes and survival after diagnosis with colon or rectal cancer

	Colon			Rectal		
	Death/Person Years	HRR[1]	(95% CI)	Death/Person Years	HRR	(95% CI)
TLR2 (rs5743704)						
CC	269 / 5869	1.00		137 / 3017	1.00	
CA/AA	28 / 511	1.89	(1.26, 2.83)	13 / 236	0.76	(0.41, 1.39)
TLR2 (rs5743708)						
GG	276 / 6036	1.00		146 / 3142	1.00	
GA/AA	22 / 346	1.74	(1.12, 2.70)	4 / 111	0.86	(0.31, 2.36)
TLR4 (rs1554973)						
TT	167 / 3426	1.00		89 / 1846	1.00	
TC	117 / 2502	0.85	(0.66, 1.08)	52 / 1163	0.85	(0.59, 1.21)
CC	14 / 455	0.55	(0.31, 0.98)	9 / 245	1.00	(0.48, 2.11)
TLR4 (rs5030728)						
GG	138 / 3119	1.00		81 / 1708	1.00	
GA	128 / 2726	1.21	(0.95, 1.55)	60 / 1317	1.00	(0.70, 1.42)
AA	32 / 535	1.52	(1.02, 2.25)	9 / 228	0.82	(0.40, 1.67)

Overall 5 year survival; adjusted for age, center, race, AJCC stage, and sex.