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Original Article

Tumor necrosis factor-related genes and colon and rectal cancer

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Abstract: Tumor necrosis factor-α (TNF) is a promoter of inflammation. Genes in the TNF pathway include tumor necrosis factor receptor superfamily member 1A (*TNFRSF1A*), TNF receptor-associated factor 2 (*TRAF2*), mitogen activated protein kinase 8 (*MAPK8*), 14 (*MAPK14*), and mitogen activated protein kinase kinase kinase 7 (*MAP3K7*), nuclear factor of activated-T-5 (*NFAT5*) cells and NFAT activated protein with ITAM motif 1 (*NFAM1*). Data from population-based studies of colon cancer (cases=1,555; controls=1,956) and rectal cancer (cases=754; controls=959) were used. We observed that *MAP3K7* rs13208824 was associated with reduced colon cancer risk (OR 0.83, 95% CI 0.71, 0.98 dominant model), *TNF* rs1800630 was associated with an increased colon cancer risk (OR 1.19 95% CI 1.03, 1.38 for CA/AAvsCC), and *TNFRSF1A* rs4149570 was associated with reduced risk (OR 0.79 95% CI 0.64, 0.96 TTvsGG). For rectal cancer *MAPK8* rs10508901 was associated with increased risk (OR 1.45 95% CI 1.05, 1.99 AA vs CC/CA; *NFAT5* (rs12447326 and rs16959025) was associated with a 40% reduced risk for the recessive model. Aspirin/NSAID interacted with *MAP3K7* (colon cancer) and with *MAPK14*, *NFAT5*, and *TRAF2* (rectal cancer); smoking cigarettes interacted with *NFAM1* and *NFAT2* (colon cancer) and *MAPK8*, *NFAT5*, and *TNFRSF1A* (rectal cancer); BMI interacted with *NFAM1* and *NFAT5* (colon cancer) and with *MAPK8* and *TNFRSF1A* (rectal cancer). A genotype summary score showed a threefold increased risk of dying with higher mutational load. Although few independent associations were detected, aspirin/NSAID, cigarette smoking, and BMI influenced genes in this pathway. These data suggest pathways through which TNF-signaling operates.

Keywords: Colon cancer, rectal cancer, TNF, MAPK8, MAPK14, NFAM1, NFAT5, TRAF2, TNFRSF1A

Introduction

Disease pathways that encompass inflammation are central to the etiology of colon and rectal cancer. Tumor necrosis factor-α (TNF), a pro-inflammatory cytokine, is thought to be one of the most important promoters of inflammation that stimulates cell proliferation and induces cell differentiation. TNF also is thought to be a modulator of insulin resistance in obesity and chronic inflammation conditions and has been reported to inhibit insulin-induced glucose uptake by targeting components of the insulin signaling cascade [1-5]. TNF mediates cell survival and apoptosis through TNF receptors by activating at least two major signaling pathways, NFκB and the p38 mitogen-activated protein (MAP) kinase pathway. NFκB activity has been associated with resistance of tumors to cytokine-induced apoptosis [6-8], helping cancer cells escape the immune response. MAP kinase signaling cascades are essential to stress and inflammatory response and play a key role in cell response to environmental stress [9, 10].

Tumor necrosis factor receptor superfamily member 1A (TNFRSF1A) is a major receptor for TNF-alpha that activates the transcription factor NFκB, mediates apoptosis, and functions as a regulator of inflammation. TNF receptor-associated factor 2 (TRAF2) is a member of the TRAF protein family that interacts with TNF receptors. TRAF2 is required for TNF-alpha activation of mitogen activated protein kinase 8 (MAPK8 alias JNK1) as well as NFκB and therefore is thought to influence the apoptotic effects of TNF. MAP3K7, also known as TAK1 and TGF1a, mediates many intracellular actions of

pro-inflammatory cytokines such as TNF. Once activated, MAP3K7 initiates a cascade of signaling events that include activation of NFκB, MAPK8, MAPK14 (p38), and other inflammatory genes [11]. MAPK14 is a key element in inflammatory response; when inactivated it can block production of pro-inflammatory cytokines, however when activated it increases expression of vascular cell adhesion molecules [12]. Another element in linking inflammation to cancer is nuclear factor of activated T (NFAT) cells and NFAT activated protein with ITAM motif 1 (NFAM1) which increases TNF promoter transcription [7, 13, 14].

In this study we evaluate genetic variation in *TNF*, *TNFRSF1A*, *TRAF2*, *MAP3K7*, *MAPK8*, *MAPK14*, *NFAT5,* and *NFAM1* as contributors to risk of colon and rectal cancer. In addition to their independent association, we evaluate their interaction with key lifestyle factors that may be associated with this pathway, aspirin/NSAID use, cigarette smoking, and BMI. We also assess the impact of these genes on survival after diagnosis.

Methods

Two population-based study populations of colon and rectal cancer that used identical data collection methods are included. The colon cancer study included cases (n=1,593) and controls (n=1,994) identified between October 1, 1991 and September 30, 1994 [15] living in the Twin Cities Metropolitan Area, Kaiser Permanente Medical Care Program of Northern California (KPMCP) and a seven-county area of Utah. The second study of the rectosigmoid junction or rectal cancer included cases (n=790) and controls (n=999) 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 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, physical activity, medical history, and cigarette smoking history, regular use of aspirin and nonsteroidal anti-inflammatory drugs, and body size. Measured height and weight recalled during the referent year was used to calculate body mass index (BMI).

TagSNP selection and genotyping

DNA was extracted from whole blood and immortalized cell lines. Genes were selected based on biological function within the TNF candidate pathway. TagSNPs were 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 analysis for that specific marker. In general, functionality of specific tagSNPs was unknown.

Statistical method

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, and sex. Analyses were run for the co-dominant, recessive, and dominant models and based on those results, the most appropriate model was determined. Analysis for interaction was based on tagSNPs within each gene. Lifestyle variables were selected because of

Table 1. Description of study population

		Colon		Rectal	
		Control n (%)	Case n (%)	Control n (%)	Case n (%)
Age	30-39	40 (2.04)	23 (1.48)	21 (2.19)	19 (2.52)
	40-49	128 (6.54)	102 (6.56)	101 (10.53)	96 (12.73)
	50-59	326 (16.67)	290 (18.65)	243 (25.34)	196 (25.99)
	60-69	673 (34.41)	538 (34.60)	329 (34.31)	250 (33.16)
	70-79	789 (40.34)	602 (38.71)	265 (27.63)	193 (25.60)
Center	Utah	378 (19.33)	249 (16.01)	365 (38.06)	274 (36.34)
	KPMCP	787 (40.24)	744 (47.85)	594 (61.94)	480 (63.66)
	Minnesota	791 (40.44)	562 (36.14)	0 (0)	0 (0)
Race/ Ethnicity	NHW	1828 (93.46)	1428 (91.83)	824 (85.92)	625 (82.89)
	Hispanics	75 (3.83)	59 (3.79)	63 (6.57)	61 (8.09)
	Black	53 (2.71)	68 (4.37)	43 (4.48)	29 (3.85)
	Asian	0	0	29 (3.02)	39 (5.17)
Sex	Male	1047 (53.53)	870 (55.95)	541 (56.41)	451 (59.81)
	Female	909 (46.47)	685 (44.05)	418 (43.59)	303 (40.19)
AJCC Stage	1		469 (30.14)		382 (50.53)
	2		406 (26.09)		124 (16.40)
	3		374 (24.04)		176 (23.28)
	4		128 (8.23)		57 (7.54)
	Unknown		179 (11.50)		17 (2.25)

their biological plausibility for involvement in this candidate pathway and included recent use of aspirin or NSAIDs, cigarette smoking (recent or not recent smoker), and BMI of kg/m2. P values for interaction were determined using a likelihood-ratio test comparing a full model that included an ordinal interaction term with a reduced model without an interaction term. Hazard rate ratios (HRR) were calculated based on months of survival after diagnosis adjusting for age, sex, race, center, tumor molecular phenotype, and AJCC stage. Survival and stage data were obtained from local tumor registries. 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.

To summarize risk associated with multiple variants across the pathway we created a summary score that was based on all at-risk genotypes identified from multiple regression models for colon and rectal cancer. The score for each SNP was based on the inheritance model and its associated risk. For the co-dominant or additive model a score of zero, one, or two was assigned directly related to the number of high-risk alleles, while scores of zero or two were assigned for the dominant and recessive models. After assigning a score for each SNP, the scores were summed across SNPs to generate an individual summary score. The score variable was categorized based on the frequency distribution within the study population.

Adjusted multiple-comparison p values, taking into account tagSNPs within the gene, were estimated using the methods by Conneely and Boehnke [18] via R version 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria). Wald p values from the original models and interaction p values based on likelihood-ratio tests were used to estimate multiple comparison adjusted p values. 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

The study population is described in **Table 1**. The majority of participants were male, over 60 at the time of diagnosis, and non-Hispanic white

(NHW). Hispanic participants represented the second largest ethnic group at roughly 4% of the colon cancer population and 6.5 to almost 8% of the rectal cancer study population.

We detected few statistically significant associations between any of the genes assessed and risk of either colon or rectal cancer (**Table 2**). For colon cancer we observed that *MAP3K7* rs13208824 was associated with reduced risk for the dominant model (OR 0.83, 95% CI 0.71, 0.98; Wald p value 0.0234 pACT 0.1201). TNF rs1800630 was associated with a slight increased risk of colon cancer (OR 1.19 95% CI 1.03, 1.38 for CA/AA genotypes relative to CC; Wald p value 0.0202; pACT 0.0326) and rs4149570 of *TNFRSF1A* was associated with a slight reduced risk for the TT genotype relative to GG (OR 0.79 95% CI 0.64, 0.96; Wald p value 0.0207; pACT 0.0815). For rectal cancer *MAPK8* rs10508901 was significantly associated with risk. Having the AA genotype relative to the CC/CA slightly increased risk (OR 1.45 95% CI 1.05, 1.99; Wald p value 0.0224; pACT 0.0577). Two SNPs of *NFAT5* (rs12447326 and rs16959025) were associated with a statistically significant 40% reduced risk of rectal cancer for the recessive model of inheritance (Wald p values 0.0029 and 0.0251 and pACT values 0.0174 and 0.1163 for rs12447326 and rs16959025 respectively).

We observed several significant interactions between aspirin/NSAID use, cigarette smoking and BMI for SNPs within the candidate genes (**Table 3**). For colon cancer, *MAP3K7* rs13208824 interacted with recent aspirin/NSAID use, with those having the CA/AA genotype having reduced risk even among those without use of aspirin/NSAIDs. Both *NFAM1* rs13055337 and *NFAT5* rs8049728 interacted with smoking and *NFAM1* rs9623589 and *NFAT5* rs9889219 interacted significantly with BMI. Recent smokers were at increased risk only if they had the *NFAM1* CG/GG genotypes while those with the TG/GG rs9623589 genotypes were associated with less risk if they had a BMI of 30 or more than were those with the TT genotype. For *NFAT5*, the GG genotype had the greatest increased risk among smokers and those with a BMI of 30 or more. For rectal cancer, *MAPK14* rs10807156 and rs851011 and *TRAF2* rs2784075 interacted with aspirin/NSAID use; MAPK8 rs4838590, *TNFRSF1A* rs4149584, and *NFAT5* rs39999 interacted significantly with smoking status; *MAPK8* rs10508901 and *TNFRSF1A* rs4149584 interacted significantly with BMI. In several instances the numbers in specific cells were small and although findings were statistically significant they should be viewed as preliminary in need of validation by other studies.

NFAM1 rs1057157 and *NFAT5* rs8049728 and rs9889219 were associated with survival for colon cancer (**Table 4**). The summary score for having all three recessive genotypes showed a HRR of 3.12 (95% CI 1.64, 5.95). *MAPK14* rs10807156 was associated with survival after diagnosis with rectal cancer (HRR for AA vs TT/TA genotypes 1.73 95% CI 1.03, 2.91). Likewise, *NFAM1* rs13055337, *NFAT5* rs39999, and *TRAF2* rs2784075 and rs7027246 were associated with survival. The summary score HRR was 2.97 (95% CI 1.41, 6.26) for having six or more high-risk genotypes.

Discussion

We detected few significant associations between the genes assessed and risk of either colon or rectal cancer. Significant associations detected, including *TNF*, *TNFRSF1A*, and *MAP3K7* for colon cancer and *MAPK8* and *NFAT5* for rectal cancer, were generally weak and were not significant after adjustment for multiple comparisons. It should be noted that the OR for *TNF* for rectal cancer was identical to that observed for colon cancer, although the confidence intervals encompassed 1.00 given the smaller number of rectal cancer study cases and controls. These genes also had minimal impact on survival, with only *NFAT5* associated with survival after diagnosis with both colon and rectal cancer and *TRAF2* associated with survival after diagnosis with rectal cancer. Perhaps the most interesting findings come from interaction with lifestyle factors hypothesized as influencing the susceptibility of these genes.

Aspirin/NSAID use interacted with *MAP3K7* to alter risk associated with colon cancer and *MAPK14* and *TRAF2* interacted with aspirin/NSAIDs to alter risk associated with rectal cancer. The TNF-signaling pathway is a key promoter of inflammatory response. Anti-inflammatory drugs such as aspirin and NSAIDs are likely lifestyle factors to modify genetic risk in inflammation-related pathways. *MAP3K7* is a key mediator of proinflammatory cytokines [11]

Table 2. Associations between SNPs in candidate genes and colon and rectal cancer

							Colon		Rectal	
Gene	Alias	Location	tagSNP	Major/Minor Allele	MAF[1]	FDR HWE[1]	Heterozygote OR (95%CI)[2]	Homozygote Rare OR (95%CI)	Heterozygote OR (95% CI)	Homozygote Rare OR (95%CI)
MAP3K7	*TAK1*	6q16.1-q16.3	rs205342	A /G	0.28	0.58	1.16(1.01,1.33)	0.95(0.74,1.21)	0.99(0.81,1.22)	1.11(0.77,1.59)
	TGF1a		rs711267	A /G	0.28	1.00		0.77(0.59,1.00)	0.98(0.80,1.21)	1.00(0.70,1.44)
			rs13208824	C /A	0.14	1.00	0.83(0.71,0.98)		1.03(0.81,1.29)	1.77(0.79,3.93)
			rs1144159	T /C	0.15	1.00	1.04(0.89,1.21)	1.10(0.71,1.71)	1.03(0.82,1.30)	1.16(0.57,2.35)
			rs3799912	A /G	0.12	0.93	1.04(0.88,1.23)	0.73(0.41,1.29)	1.00(0.78,1.27)	0.91(0.40,2.07)
			rs150117	A /T	0.32	0.70	1.04(0.90,1.19)	0.85(0.67,1.07)	0.94(0.77,1.15)	1.16(0.84,1.59)
MAPK8	*JNK*	10q11.22	rs10857565	G /A	0.23	0.68	0.95(0.82,1.09)	1.23(0.90,1.69)	1.17(0.95,1.43)	1.13(0.70,1.81)
	JNK1		rs10508901	C /A	0.33	0.68	0.97(0.84,1.12)	1.12(0.90,1.41)		1.45(1.05,1.99)
	SAPK1		rs4838590	C /A	0.43	1.00	1.02(0.87,1.19)	1.12(0.92,1.36)	1.06(0.85,1.32)	1.16(0.88,1.52)
MAPK14	*CSBP1*	6p21.3-p21.2	rs13196204	T /G	0.17	1.00	1.04(0.90,1.21)	1.39(0.95,2.04)	1.20(0.97,1.49)	0.93(0.52,1.67)
	CSBP2		rs10807156	T /A	0.21	1.00	0.96(0.83,1.11)	0.84(0.60,1.17)	1.09(0.89,1.34)	0.99(0.65,1.50)
	CSPB1		rs3804454	A /C	0.21	0.85	0.98(0.84,1.13)	0.86(0.62,1.18)	0.98(0.80,1.21)	1.08(0.69,1.70)
	RK		rs17714205	C /T	0.11	0.58	1.01(0.85,1.21)	1.04(0.58,1.85)	1.04(0.81,1.34)	1.33(0.63,2.81)
	EXIP		rs3730327	A /G	0.10	1.00	0.94(0.79,1.12)	0.75(0.39,1.45)	0.97(0.76,1.24)	0.79(0.30,2.05)
	p38		rs851011	T /C	0.14	1.00	0.96(0.82,1.12)	1.23(0.76,2.00)	0.96(0.76,1.22)	0.80(0.37,1.71)
	SAPK2A		rs851007	T /C	0.47	1.00	1.00(0.86,1.18)	0.96(0.79,1.16)	0.93(0.74,1.18)	0.87(0.66,1.15)
	p38alpha		rs851006	G /A	0.25	1.00	0.94(0.82,1.09)	1.17(0.88,1.56)	0.96(0.78,1.18)	1.06(0.70,1.60)
NFAM1	*CTA-126B4.4*	22q13.2	rs1057157	A /G	0.36	1.00	1.12(0.98,1.29)		0.89(0.72,1.10)	1.02(0.75,1.37)
	CNAIP		rs2413687	C /T	0.46	1.00	0.91(0.78,1.06)	0.91(0.75,1.10)	1.00(0.81,1.25)	1.15(0.88,1.52)
	FLJ40652		rs13055337	C /G	0.17	0.96	1.03(0.89,1.19)	1.10(0.76,1.61)	0.87(0.70,1.08)	0.98(0.58,1.66)
	bK126B4.4		rs12160838	G /A	0.16	0.97	1.11(0.96,1.29)	1.19(0.78,1.81)	0.90(0.73,1.12)	1.04(0.58,1.88)
			rs2142831	A /G	0.38	0.85	1.07(0.93,1.24)	1.07(0.88,1.31)	0.95(0.77,1.17)	0.99(0.74,1.32)
			rs735537	A /G	0.47	0.76	1.03(0.88,1.21)	0.99(0.82,1.20)	1.05(0.84,1.32)	0.92(0.70,1.21)
			rs4822127	C /G	0.25	1.00	0.87(0.76,1.00)		0.93(0.76,1.13)	0.91(0.59,1.40)
			rs9623589	T /G	0.31	1.00	0.89(0.78,1.02)		0.98(0.80,1.19)	0.89(0.63,1.27)
			rs9611791	G /A	0.39	0.68	1.01(0.87,1.17)	0.90(0.74,1.11)	1.08(0.88,1.33)	1.14(0.86,1.53)

			rs8141826	A /G	0.43	1.00	1.13(0.98,1.31)		0.86(0.69,1.07)	0.91(0.70,1.19)
			rs4822129	G /A	0.20	1.00	0.88(0.76,1.01)		1.00(0.81,1.24)	0.93(0.56,1.55)
			rs9620047	A /G	0.14	0.95	1.01(0.86,1.18)	0.99(0.64,1.54)	0.96(0.77,1.20)	1.09(0.60,1.98)
NFAT5	*KIAA0827*	16q22.1	rs12447326	C /T	0.27	0.57	1.06(0.92,1.22)	1.07(0.83,1.38)		0.60(0.43,0.84)
	NF-AT5		rs39999	G /C	0.15	0.85	0.92(0.79,1.08)	0.89(0.58,1.38)	1.02(0.81,1.30)	1.05(0.53,2.07)
	NFATL1		rs8049728	G /T	0.44	0.90	0.99(0.84,1.15)	1.11(0.92,1.34)	1.09(0.87,1.37)	0.93(0.71,1.22)
	NFATZ		rs244415	G /A	0.42	1.00	0.98(0.85,1.14)	0.99(0.81,1.21)	1.00(0.81,1.23)	1.03(0.78,1.38)
	OREBP		rs2304526	A /G	0.16	0.97	1.05(0.90,1.22)	0.91(0.62,1.35)	1.05(0.85,1.30)	1.27(0.74,2.18)
	TONEBP		rs16959025	T /G	0.19	0.54	1.06(0.91,1.22)	1.08(0.75,1.56)		0.57(0.35,0.93)
			rs9889219	G /A	0.25	0.97	0.96(0.83,1.11)	1.03(0.79,1.34)	0.99(0.81,1.22)	0.98(0.66,1.45)
TNF	*TNFA*	6p21.3	rs1799964	T /C	0.21	0.95	1.10(0.96,1.27)	1.29(0.94,1.76)	1.09(0.89,1.34)	1.21(0.78,1.87)
	TNFSF2		rs1800630	C /A	0.15	0.72	1.19(1.03,1.38)		1.19(0.97,1.47)	
TNFRSF1A	*FPF*	12p13.2	rs4149584	G /A	0.02	0.77	0.97(0.70,1.34)		1.07(0.63,1.81)	
	TNFAR		rs4149578	G /A	0.09	1.00	1.02(0.86,1.22)	0.97(0.51,1.85)	1.10(0.85,1.40)	1.18(0.55,2.52)
	TNFR		rs4149577	T /C	0.49	0.86	0.93(0.79,1.09)	0.85(0.71,1.03)	1.10(0.87,1.37)	0.96(0.74,1.25)
	TNF-R1		rs4149576	G /A	0.42	0.68	1.08(0.93,1.25)	1.06(0.87,1.30)	1.12(0.90,1.39)	1.04(0.78,1.38)
	p55; p60		rs4149570	G /T	0.41	1.00	0.91(0.79,1.06)	0.79(0.64,0.96)	1.06(0.86,1.31)	1.11(0.83,1.49)
TRAF2	*MGC*	9q34	rs2784075	G /A	0.23	0.81	0.93(0.80,1.07)	0.96(0.72,1.29)	0.98(0.80,1.20)	1.15(0.75,1.75)
			rs7027246	G /A	0.26	0.68	0.96(0.83,1.11)	0.82(0.63,1.07)	0.99(0.81,1.21)	1.09(0.73,1.61)
			rs4880073	G /A	0.40	0.68	1.08(0.93,1.25)	1.01(0.83,1.24)	1.10(0.89,1.37)	0.93(0.70,1.24)
			rs908831	A /G	0.40	0.93	1.02(0.88,1.18)	0.90(0.74,1.10)	0.86(0.70,1.07)	0.97(0.72,1.29)

[1]Minor Allele Frequency (MAF) and FDR-adjusted Hardy-Weinberg Equilibrium (FDR HWE) based on white control population; [2]Odds Ratio (OR) and 95% Confidence Intervals (CI) Dominant model listed in 'Heterozygote OR' column only; recessive model listed in 'Homozygote Rare OR' column only.

Table 3. Interaction between aspirin/NSAIDs, cigarette smoking and BMI and SNPs in candidate genes.

	Controls N	Cases N	OR[1]	(95% CI)	Controls N	Cases N	OR	(95% CI)	Controls N	Cases N	OR	(95% CI)
Colon	No Recent Aspirin/NSAID Use				Recent Aspirin NSAID Use							
MAP3K7 (rs13208824)												
CC	849	844	1.00		617	369	0.61	(0.52, 0.71)				
CA/AA	287	209	0.75	(0.61, 0.91)	187	116	0.63	(0.49, 0.81)				
P Interaction 0.0496; pACT 0.2366												
	Non-Smoker / Non-Recent Smoker				Recent Smoker							
NFAM1 (rs13055337)												
CC	1084	841	1.00		234	189	1	(0.81, 1.24)				
CG/GG	525	393	0.95	(0.81, 1.12)	112	129	1.46	(1.11, 1.91)				
P Interaction 0.0192; pACT 0.1529												
NFAT5 (rs8049728)												
GG	512	364	1.00		99	109	1.5	(1.11, 2.04)				
GT	790	592	1.04	(0.88, 1.24)	167	145	1.17	(0.90, 1.52)				
TT	306	278	1.24	(1.00, 1.53)	80	64	1.07	(0.75, 1.53)				
P Interaction 0.0212; pACT 0.1124												
	BMI <25				BMI 25-29				BMI >=30			
NFAM1 (rs9623589)												
TT	366	228	1.00		386	345	1.44	(1.15, 1.80)	159	195	1.93	(1.47, 2.52)
TG/GG	391	276	1.14	(0.91, 1.43)	410	285	1.1	(0.88, 1.38)	239	221	1.47	(1.15, 1.89)
P Interaction 0.0181; pACT 0.1439												
NFAT5 (rs9889219)												
GG	461	283	1.00		417	348	1.35	(1.09, 1.66)	217	249	1.84	(1.45, 2.33)
GA	247	184	1.21	(0.95, 1.54)	323	238	1.19	(0.95, 1.49)	148	134	1.45	(1.09, 1.91)
AA	49	37	1.20	(0.76, 1.88)	53	44	1.33	(0.86, 2.04)	34	33	1.52	(0.92, 2.51)
P Interaction 0.0383; pACT 0.1898												
Rectal	No Recent Aspirin/NSAID Use				Recent Aspirin NSAID Use							
MAPK14 (rs10807156)												
TT	329	276	1.00		254	170	0.81	(0.63, 1.04)				
TA	163	167	1.21	(0.93, 1.59)	146	91	0.74	(0.54, 1.01)				
AA	29	33	1.32	(0.78, 2.23)	27	10	0.44	(0.21, 0.93)				
P Interaction 0.0401; pACT 0.1945												
MAPK14 (rs851011)												
TT	388	384	1.00		340	196	0.59	(0.47, 0.74)				
TC/CC	133	93	0.73	(0.54, 0.99)	88	75	0.87	(0.62, 1.22)				

P Interaction 0.0032; pACT 0.0218															
TRAF2 (rs2784075)															
GG	297	298	1.00			274	152	0.56	(0.43,	0.72)					
GA	198	150	0.75	(0.58,	0.98)	131	104	0.8	(0.59,	1.08)					
AA	26	29	1.10	(0.63,	1.92)	23	15	0.66	(0.34,	1.30)					
P Interaction 0.0357; pACT 0.1022															
	Non-Smoker / Non-Recent Smoker					Recent Smoker									
MAPK8 (rs4838590)															
CC	275	180	1.00			42	56	1.99	(1.28,	3.11)					
CA	382	289	1.17	(0.92,	1.49)	77	68	1.32	(0.91,	1.93)					
AA	152	133	1.35	(1.00,	1.82)	31	24	1.15	(0.65,	2.04)					
P Interaction 0.0152; pACT 0.0399															
NFAT5 (rs39999)															
GG	628	449	1.00			111	128	1.57	(1.18,	2.09)					
GC/CC	181	153	1.20	(0.94,	1.54)	39	20	0.71	(0.41,	1.23)					
P Interaction 0.0025; pACT 0.0154															
TNFRSF1A (rs4149584)															
GG	785	578	1.00			141	145	1.36	(1.05,	1.76)					
GA/AA	23	23	1.39	(0.77,	2.51)	9	3	0.46	(0.12,	1.71)					
P Interaction 0.0435; pACT 0.1677															
	BMI <25					BMI 25-29					BMI >=30				
MAPK8 (rs10508901)															
CC/CA	275	221	1.00			377	268	0.85	(0.67,	1.09)	220	172	0.94	(0.72,	1.23)
AA	36	22	0.78	(0.44,	1.36)	31	34	1.34	(0.80,	2.26)	15	32	2.65	(1.40,	5.04)
P Interaction 0.0023; pACT 0.0064															
TNFRSF1A (rs4149584)															
GG	296	236	1.00			395	295	0.91	(0.72,	1.15)	230	192	1.01	(0.78,	1.31)
GA/AA	15	7	0.58	(0.23,	1.46)	12	6	0.63	(0.23,	1.71)	5	12	3.06	(1.06,	8.83)
P Interaction 0.0194; pACT 0.0798															

[1]Odds Ratios (OR) and 95% Confidence Intervals (CI) adjusted for age, sex, center, race/ethnicity

Table 4. Genetic variation associated with survival after diagnosis with colon and rectal cancer

	Death/ Person Years	HRR[1]	(95% CI)
		Colon	
NFAM1 (rs1057157)			
AA/AG	262 / 7001	1.00	
GG	47 / 1147	1.40	(1.02, 1.93)
NFAT5 (rs8049728)			
GG/GT	246 / 6265	1.00	
TT	63 / 1883	0.75	(0.56, 0.99)
NFAT5 (rs9889219)			
GG/GA	291 / 7533	1.00	
AA	18 / 614	0.51	(0.31, 0.82)
Summary Score			
(0-0)	14/532	1.00	
(2-2)	38/1148	2.29	(1.23, 4.27)
(4-4)	225/5605	2.39	(1.38, 4.13)
(6-6)	32/863	3.12	(1.64, 5.95)
P Trend	0.0006		
		Rectal	
MAPK14 (rs10807156)			
TT/TA	153 / 4044	1.00	
AA	17 / 240	1.73	(1.03, 2.91)
NFAM1 (rs13055337)			
CC	132 / 2962	1.00	
CG/GG	39 / 1319	0.68	(0.47, 0.98)
NFAT5 (rs39999)			
GG	124 / 3318	1.00	
GC/CC	47 / 972	1.56	(1.11, 2.21)
TRAF2 (rs2784075)			
GG/GA	157 / 4048	1.00	
AA	14 / 241	1.89	(1.08, 3.31)
TRAF2 (rs7027246)			
GG/GA	155 / 4009	1.00	
AA	16 / 280	1.76	(1.03, 2.99)
Summary Score			
(0-0)	21/876	1.00	
(2-2)	90/2427	1.83	(1.12, 2.99)
(4-4)	49/754	2.77	(1.64, 4.67)
(6-10)	11/233	2.97	(1.41, 6.26)
P Trend	<.0001		

[1]Hazard Rate Ratio (HRR) and 95% Confidence Intervals (CI) adjusted for age, center, race, sex, AJCC stage and tumor molecular phenotype.

and is an important initiator of signaling events that include NFκB, MAPK14 (p38 alpha), and MAPK8 (JNK1). TRAF2 has been shown to be necessary for MAPK14 activation by TNF [19], and aspirin has been shown to inhibit innate immune signaling by inducing degradation of TRAF2 [20]. The biological function of MAP3K7, MAPK14, and TRAF2 early in the inflammation process may be central to the associations observed. While it is unclear why different genes would be important for colon and rectal cancer, studies have shown that MAPK14 mediates inflammatory response and its ability to influence the inflammation process is cell specific [21]. Additionally it is commonly observed that the two cancer sites have unique risk factors and etiology may react differently due to different factors initiating the inflammation process.

We observed that the same genes interacted with both cigarette smoking and BMI. While this pattern of association is of interest, different genes were important for colon than for rectal cancer. For colon cancer, *NFAM1* and *NFAT5* interacted with both cigarette smoking and BMI, while *MAPK8* and *TNFSF1A* interacted with both smoking and BMI to alter rectal cancer risk. Reactive oxygen species associated with cigarette smoking has been shown to be key mediators of many cellular responses to TNF including apoptosis and insulin signaling [22]. TNF operates through its receptors; blocking TNF leads to increased insulin sensitivity [23]. *TNFSF1A* is a major receptor of TNF and may be involved in insulin resistance associated with TNF [3]. MAPK8 has been shown to play a role in glucose and lipid metabolism and has been linked to insulin resistance [24]. NFAM1 works with NFAT5 and increases TNF promoter transcription [7]. Although these associations have not previously been reported, there is biological rationale to support their association.

The study has many strengths. The data included in this study is extensive including genetic data as well as lifestyle data and survival information. Because of this, we have the ability to thoroughly evaluate genes to determine not only broad independent associations, but also to help identify intervening factors that may modify associations with genes. The study is hypothesis driven, examining specific factors hypothesized to influence genetic susceptibility. Because many comparisons were made, we adjusted for these comparisons and report adjusted pACT values even though this was a candidate pathway approach with only hypothesized interactions being assessed. A limitation is lack of information on functionality of polymorphisms examined in this study. Our results provide insight for follow-up studies to evaluate these polymorphisms to determine their functionality.

In summary, we observed few associations between the genes evaluated and colon or rectal cancer risk. However, as hypothesized, NSAIDs, cigarette smoking, and BMI interacted significantly with several of these genes. These associations need reproduction in other studies.

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