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MAP kinase genes and colon and rectal cancer

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Mitogen-activated protein kinase (MAPK) pathways regulate many cellular functions including cell proliferation, differentiation, migration and apoptosis. We evaluate genetic variation in the c-Jun-N-terminal kinases, p38, and extracellular regulated kinases 1/2 MAPK-signaling pathways and colon and rectal cancer risk using data from population-based case-control studies (colon: *n* = 1555 cases, 1956 controls; rectal: *n* = 754 cases, 959 controls). We assess 19 genes (*DUSP1, DUSP2, DUSP4, DUSP6, DUSP7, MAP2K1, MAP3K1, MAP3K2, MAP3K3, MAP3K7, MAP3K9, MAP3K10, MAP3K11, MAPK1, MAPK3, MAPK8, MAPK12, MAPK14* and *RAF1*). *MAP2K1* rs8039880 [odds ratio (OR) = 0.57, 95% confidence interval (CI) = 0.38, 0.83; GG versus AA genotype] and *MAP3K9* rs11625206 (OR = 1.41, 95% CI = 1.14, 1.76; recessive model) were associated with colon cancer (P_{adj} value < 0.05). *DUSP1* rs322351 (OR = 1.43, 95% CI = 1.09, 1.88; TT versus CC) and *MAPK8* rs10857561 (OR = 1.48, 95% CI 1.08, 2.03; AA versus GG/GA) were associated with rectal cancer (P_{adj} < 0.05). Aspirin/non-steroidal anti-inflammatory drug, cigarette smoking and body mass index interacted with several genes to alter cancer risk. Genetic variants had unique associations with *KRAS*, *TP53* and CIMP+ tumors. *DUSP2* rs1724120 [hazard rate ratio (HRR) = 0.72, 95%CI = 0.54, 0.96; AA versus GG/GA), *MAP3K10* rs112956 (HRR = 1.40, 95% CI = 1.10, 1.76; CT/TT versus CC) and *MAP3K11* (HRR = 1.76, 95% CI 1.18, 2.62 TT versus GG/GT) influenced survival after diagnosis with colon cancer; *MAP2K1* rs8039880 (HRR = 2.53, 95% CI 1.34, 4.79 GG versus AG/GG) and *Raf1* rs11923427 (HRR = 0.59 95% CI = 0.40, 0.86; AA versus TT/TA) were associated with rectal cancer survival. These data suggest that genetic variation in the MAPK-signaling pathway influences colorectal cancer risk and survival after diagnosis. Associations may be modified by lifestyle factors that influence inflammation and oxidative stress.

Introduction

Mitogen-activated protein kinase (MAPK) pathways regulate many cellular functions including cell proliferation, differentiation, migration and apoptosis (1). They are activated by a variety of stimuli and phosphorylate transcription factors, kinases and other enzymes, and influence gene expression, metabolism, cell division, morphology and survival. Each MAPK pathway is a three-tiered cascade that includes a MAP kinase kinase kinase (MAP3K, MEKK or MKKK), a Map kinase kinase (MAP2K, MEK or MKK) and the MAP kinase (MAPK). MAPK are attenuated by dual-specificity MAPK phosphatases (MKPs or DUSP). Three of the major MAPK pathways are extracellular regulated kinases 1 and 2 (ERK1/2), c-Jun-N-terminal kinases (JNKs) and p38. ERK5 and ERK3/ERK4 are less well-studied pathways (2).

Abbreviations: AJCC, American Joint Committee on Cancer; BMI, body mass index; CI, confidence interval; CRC, colorectal cancer; HRR, hazard rate ratio; KPMCP, Kaiser Permanente Medical Care Program of Northern California; LD, linkage disequilibrium; MAF, minor allele frequency; MAPK, mitogen-activated protein kinase; NSAID, non-steroidal anti-inflammatory drug; OR: odds ratio; SNPs, single-nucleotide polymorphisms.

ERK1 and ERK2 regulate proliferation, differentiation and meiosis and are activated by stimuli such as growth factors and cytokines. Raf, a MAP kinase kinase kinase, is involved in the ERK1/2 pathway as the initial responder to growth factors and cytokines (1). Ras has been showed to be mutated in tumors associated with *ERK1* and *ERK2* (3). The JNK pathway is involved in regulating responses to stress, inflammation and apoptosis and are activated by radiation, environmental stresses and growth factors. Studies have shown the JNK pathway being involved in development of obesity and type 2 diabetes (4,5). The *p38* MAPKs are involved in autoimmunity in humans and are activated by chemical stresses, hormones, cytokines including interleukin-1 and tumor necrosis factor, and shock (1,2). The p38 targets several transcription factors, including nuclear factor-kappaB and *TP53* (2).

Few epidemiological studies have evaluated the risk associated with genetic variation in MAPK-signaling pathways and cancer. However, the MAPK-signaling pathways have been identified as one of the most strongly associated gene markers to colorectal cancer (CRC) from a genome-wide association study conducted in Germany (6). Seven MAPK genes were identified as being important for CRC in that study. A study by Barault *et al.* has shown that somatic mutations in MAPK correlated with poor survival after diagnosis with CRC (7).

In this study, we evaluate genetic variation in MAPK pathways using a candidate gene approach and risk of colon and rectal cancer. We evaluate if associations are uniquely associated with specific tumor molecular phenotype and if they influence survival. Because of the activation of the MAPK pathways by inflammation, oxidative stress, hormones and growth factors, we also evaluate interaction between these single-nucleotide polymorphisms (SNPs) in these candidate genes and use of aspirin/non-steroidal anti-inflammatory drugs (NSAIDs), cigarette smoking, estrogen status in women and body mass index (BMI). Data for this study were from a large case-control study of colon and rectal cancer.

Materials and methods

Two study populations are included in these analyses. The first study, a population-based case-control study of colon cancer, included cases (*n* = 1 555 with complete genotype data) and controls (*n* = 1 956 with complete genotype data) identified between 1 October 1991 and 30 September 1994 (8) living in the Twin Cities Metropolitan Area or a seven-county area of Utah or enrolled in the Kaiser Permanente Medical Care Program of Northern California (KPMCP). The second study, with identical data collection methods, included cases with cancer of the rectosigmoid junction or rectum (*n* = 754 cases and *n* = 959 controls with complete genotype data) who were identified between May 1997 and May 2001 in Utah and at the KPMCP (9). Eligible cases were between 30 and 79 years of age at the time of diagnosis, living in the study geographic area, English speaking, mentally competent to complete the interview and with no history of CRC and no previous diagnosis of familial adenomatous polyposis, ulcerative colitis or Crohn's disease. Cases who did not meet these criteria were ineligible as were individuals who were not black, white or Hispanic for the colon cancer study because the diet questionnaire was only validated in those populations. A rapid reporting system was used to identify cases within months of diagnosis.

Controls were matched to cases by sex and by 5 years age groups. At KPMCP, controls were randomly selected from membership lists; in Utah, controls ≥65 years were randomly selected from the Health Care Financing Administration lists and controls <65 years were randomly selected from driver's license lists. In Minnesota, controls were selected from driver's license and state-identification lists. Eligibility for controls was the same as those outlined for cases; additionally, controls could not have had a previous CRC. Study details have been reported previously (10,11). All study participants provided informed consent prior to completing the study questionnaire; the study was approved by the institutional review board on human subjects at all institutions.

Interview data collection

Data were collected by trained and certified interviewers using laptop computers. All interviews were audio-taped as described previously and reviewed for quality control purposes (12). The referent period for the study was 2 years prior to diagnosis for cases and selection for controls. Detailed information was collected on diet, physical activity, medical history, reproductive history, family history of cancer, regular use of aspirin and NSAIDs, cigarette smoking history and body size. Use of aspirin and NSAIDs on a regular basis defined as at least three times a week for 1 month, the total amount of time taken and date last taken. Participants who reported having smoked at least 100 cigarettes were classified as a smoker. For those individuals, we obtained the amount usually smoked and the year first and last having smoked cigarettes; recent cigarette smoking was defined as having smoked cigarettes within the 2 years prior to diagnosis or selection. Self-reported weight for 2 years prior to diagnosis (or 5 years prior to diagnosis if 2-year self-reported weight was unknown) was used along with measured height to calculate BMI (BMI of kg/m^2). Estrogen status was determined for women based on the being pre- or post-menopausal. Women who were taking hormone replacement therapy were considered estrogen positive along with those women who reported being pre-menopausal.

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 >5 years of follow-up.

Tumor marker data

We have previously evaluated tumors for CIMP, MSI, *TP53* and *KRAS* mutations (13–16) and were, therefore, able to evaluate variation in the specified genes in relation to molecularly defined subsets of CRC. Details for methods used to evaluate epigenetic and genetic changes have been described (13–16). Because of the rarity of MSI+ rectal tumors (17), we did not evaluate MSI in rectal tumors.

TagSNP selection and genotyping

TagSNPs were selected for genes *DUSP1 (2), DUSP2 (1), DUSP4 (6), DUSP6 (4), DUSP7 (1), MAPK1 (6), MAPK3 (1), RAF1 (8), MAPK8 (6), MAP3K1 (8), MAP3K3 (3), MAP3K7 (6), MAP3K9 (19), MAP3K10 (3), MAP3K11 (4), MAPK12 (3), MAPK14 (12), MAP2K1 (7), MAP3K2 (3)* and *MAPK7 (1)* using the following parameters: linkage disequilibrium (LD) blocks using a Caucasian LD map with $r^2 = 0.8$; minor allele frequency (MAF) > 0.1; range = −1500 bps from the initiation codon to +1500 bps from the termination codon and one SNP/LD bin. LD maps are included in the Supplementary Table (available at *Carcinogenesis* Online). All markers were genotyped using a multiplexed bead-array assay based on GoldenGate chemistry (Illumina, San Diego, CA). A genotyping call rate of 99.85% was attained. Blinded internal replicates represented 4.4% of the samples. The duplicate concordance rate was 100%. Two *DUSP6,* one *MAPK8*, one *MAPK12*, and the single *MAPK7* tagSNP failed. Table 2 describes tagSNPs associated with colon or rectal cancer, whereas Supplementary Table (available at *Carcinogenesis* Online) has a listing of all tagSNPs included on the platform. Genes were selected based on literature that suggested a biological function with CRC at the time the Illumina platform was created.

Statistical methods

Statistical analyses were performed for each study independently using SAS® version 9.2 (SAS Institute, Cary, NC). The LD measure, MAF and test for Hardy-Weinberg Equilibrium were calculated among white controls using the ALLELE procedure. We report odds ratios (ORs) and 95% confidence intervals (CIs) assessed from multiple logistic regression models adjusting for age, study center, race/ethnicity and sex, which were matching variables for the original studies. Further adjustment for aspirin/NSAID use, cigarette smoking and estrogen status did not influence the point estimates and, therefore, were not considered confounders of the association between SNPs and disease. All SNPs were evaluated first by comparing the heterozygote and homozygote variant to the homozygote wild-type and subsequently assessing the dominant and recessive models if those models appeared more appropriate; the best-fitting inheritance model is presented for those SNPs that were statistically significant.

Table I. Descriptive table of population

		Colon			Rectal		
		Controls	Cases	*P* value	Controls	Cases	*P* value
		n (%)	*n* (%)		*n* (%)	*n* (%)	
Age	30–39	40 (2.04)	23 (1.48)		21 (2.19)	19 (2.52)	NA
	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)	
	Mean	65	65		62	61	
Study center	Utah	378 (19.33)	249 (16.01)		365 (38.06)	274 (36.34)	NA
	KPMCP	787 (40.24)	744 (47.85)		594 (61.94)	480 (63.66)	
	Minnesota	791 (40.44)	562 (36.14)				
Race/ethnicity	NHW	1828 (93.46)	1428 (91.83)		824 (85.92)	625 (82.89)	NA
	Hispanic	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				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)	
Recent NSAID use		804 (41.44)	485 (31.53)	<0.01	428 (45.10)	271 (36.23)	<0.01
Recent smoker		346 (17.70)	318 (20.49)	0.04	150 (15.64)	148 (19.73)	0.03
Recent estrogen exposure		361 (40.84)	222 (33.08)	<0.01	249 (59.57)	161 (53.31)	0.09
AJCC stage	0/I		469 (30.16)			381 (50.53)	
	II		405 (26.05)			124 (16.45)	
	III		374 (24.05)			175 (23.21)	
	IV		128 (8.23)			57 (7.56)	
	Unknown		179 (11.51)			17 (2.25)	
Tumor markers	CIMP+		272 (17.49)			59 (7.82)	
	KRAS2 mutation		348 (22.38)			173 (22.94)	
	TP53 mutation		516 (33.18)			277 (36.74)	
	MSI+		185 (11.90)			14 (1.86)	

Table II. Descriptive table of genes associated with colon or rectal cancer

Pathway	Gene	Alias	Chromosome location	tagSNP	Major/minor allele	MAF
DUSP	*DUSP1*	*CL100, HVH1*	5q34	rs322351	C/T	0.48
		MKP-1, MKP1, PTPN10		rs881150	T/A	0.25
	DUSP2	*PAC-1, PAC1*	2q11	rs1724120	G/A	0.45
	DUSP4	*HVH2, MKP-2*	8p12–p11	rs2341674	C/T	0.16
		MKP2, TYP		rs474824	T/C	0.37
	DUSP6	*MKP3, PYST1*	12q22–q23	rs770087	T/G	0.2
ERK1/2	*MAPK1*	*ERK2*	22q11.21	rs2298432	C/A	0.38
		P42MAPK		rs9610375	G/T	0.46
		PRKM1, PRKM2,		rs8136867	A/G	0.47
				rs11913721	A/C	0.41
	MAPK3	*ERK1, PRKM3*	16p11.2	rs7698	C/T	0.07
		P44ERK1, P44MAPK				
	Raf1	*CRAF*	3p25.2	rs3729931	C/T	0.37
		Raf-1		rs9809501	T/G	0.1
		c-Raf		rs11923427	C/G	0.16
		MSV		rs11711419	A/T	0.19
				rs4684871	A/G	0.41
				rs904453	C/A	0.44
JNK	*MAPK8*	*JNK1*	10q11.22	rs10857561	G/A	0.33
		PRKM8,SAPK1		rs10857565	G/A	0.23
				rs4838590	C/A	0.43
				rs11101320	G/A	0.42
	MAP3K1[a,b]	*MAPKKK1*	5q11.2	rs16886403	T/C	0.1
		MEKK1		rs2548663	A/G	0.28
	MAP3K3[a]	*MAPKKK3*	17q23.3	rs11658329	G/C	0.28
		MEKK3		rs3785574	A/G	0.33
	MAP3K7[a]	*TAK1*	6q15	rs13208824	C/A	0.14
		Transforming growth factor		rs1144159	T/C	0.15
		β-activated kinase 1		rs3799912	A/G	0.12
				rs150117	A/T	0.32
	MAP3K9	*MLK1*	14q24.2–q31	rs11625206	C/T	0.33
				rs11844774	T/C	0.43
				rs11628333	T/C	0.36
				rs17176971	G/A	0.18
				rs4902854	C/T	0.40
				rs1034769	T/G	0.12
				rs17766621	T/C	0.35
	MAP3K10	*MLK2*	19q13.2	rs892117	T/C	0.49
		MST, MKN28 kinase		rs1129156	C/T	0.27
	MAP3K11[a]	*PTK1*	11q13.1–q13.3	rs11227234	G/T	0.26
		MLK-3, MLK3		rs1151488	A/G	0.31
				rs7116712	T/C	0.41
				rs1784223	T/C	0.34
p38	*MAPK12*	*ERK6*	22q13.33	rs2272857	G/A	0.24
		P38GAMMA, SAPK3				
	MAPK14	*p38,p38ALPHA*	6p21.3–p21.2	rs10807156	T/A	0.21
		CSBP1,		rs17714205	C/T	0.11
		MXI2,		rs851016	A/G	0.14
		SAPK2A		rs851006	G/A	0.25
	MAP2K1	*MAPKK1,MEK1*	15q22.1–q22.33	rs7181936	G/T	0.32
		MKK1,PRKMK1		rs8039880	A/G	0.19
	MAP3K2[a]	*MEKK2*	2q14.3	rs3732209	T/C	0.30

MAF based on white control population.
[a]Operates in both JNK and p38 pathways.
[b]Also regulates ERK pathway.

Lifestyle variables associated with colon and rectal cancer were evaluated because of their potential involvement in MAPK-signaling pathways. We evaluated interactions between aspirin/NSAID use, recent cigarette smoking, estrogen status and BMI. *P* values for interaction were determined using a 1-df likelihood-ratio test comparing a full model that included an interaction term to a reduced model without an interaction term.

Tumors were defined by specific molecular alterations: any *TP53* mutation, any *KRAS* mutation, MSI+, CIMP+ defined as at least two of five markers methylated or a combination of CIMP+/MSI+. As the proportion of MSI+ tumors in the rectal cases was <3% (17), we did not examine these tumor markers. Estimates of risk for molecular tumor phenotypes were made relative to controls using generalized estimating equations, assuming an independent correlation structure. We calculated the *P* for heterogeneity to determine if associations were unique to specific molecular phenotypes using a case/case comparison analysis within the LOGISTIC framework.

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 CRC were evaluated using Cox proportional hazards models to obtain multivariate hazard rate ratios (HRRs) and 95% CIs, censoring individuals when they died of causes other than CRC or were lost to follow-up. In addition to the minimal adjustments for age at diagnosis, study center, race/ethnicity and sex, we also adjusted for tumor molecular phenotype and AJCC stage to estimate HRRs.

Multiple comparison adjustments were made taking into account tagSNPs within the gene using the step-down Bonferroni correction (i.e. Holm method) based on the effective number of independent SNPs as determined using the

Table III. Associations with colon and rectal cancer

	Colon						Rectal					
	Controls	Cases	OR[a]	(95% CI)	Wald *P*	Holm *P*	Controls	Cases	OR	(95% CI)	Wald *P*	Holm *P*
DUSP1 (rs322351)												
CC	543	471	1		0.135	0.235	303	216	1		0.013	0.023
CT	976	761	0.91	(0.78, 1.07)			469	356	1.09	(0.87, 1.37)		
TT	437	323	0.87	(0.72, 1.05)			187	182	1.43	(1.09, 1.88)		
MAP2K1 (rs7181936)												
GG/GT	1766	1369	1.00		0.027	0.135	845	667	1		0.820	1.000
TT	189	186	1.27	(1.03, 1.58)			114	87	0.97	(0.72, 1.30)		
MAP2K1 (rs8039880)												
AA	1255	1049	1.00		0.007	0.042	611	507	1		0.216	1.000
AG	616	466	0.90	(0.78, 1.04)			312	212	0.8	(0.65, 0.99)		
GG	84	40	0.57	(0.38, 0.83)			36	35	1.12	(0.69, 1.81)		
MAP3K3 (rs11658329)												
GG	1008	744	1.00		0.035	0.087	500	392	1		0.973	1.000
GC/CC	948	811	1.16	(1.01, 1.32)			459	362	1	(0.83, 1.22)		
MAP3K3 (rs3785574)												
AA/AG	1723	1407	1.00		0.031	0.087	862	670	1		0.546	1.000
GG	233	148	0.79	(0.63, 0.98)			97	84	1.1	(0.81, 1.50)		
MAP3K7 (rs13208824)												
CC	1476	1228	1.00		0.023	0.117	732	572	1		0.613	1.000
CA/AA	480	327	0.83	(0.71, 0.98)			227	182	1.06	(0.84, 1.33)		
MAP3K9 (rs11625206)[b]												
CC/CT	1777	1364	1.00		0.002	0.022	852	678	1		0.524	1.000
TT	178	189	1.41	(1.14, 1.76)			107	76	0.9	(0.66, 1.23)		
MAP3K9 (rs11628333)[b]												
TT/TC	1720	1331	1.00		0.028	0.243	823	658	1		0.438	1.000
CC	235	223	1.25	(1.02, 1.52)			136	96	0.89	(0.68, 1.19)		
MAP3K9 (rs11844774)[c]												
TT	626	563	1.00		0.008	0.081	310	248	1		0.662	1.000
TC/CC	1329	991	0.83	(0.72, 0.95)			649	506	0.96	(0.78, 1.17)		
MAP3K10 (rs1129156)												
CC/CT	1806	1468	1.00		0.031	0.061	892	694	1		0.282	0.563
TT	147	86	0.74	(0.56, 0.97)			64	60	1.22	(0.85, 1.77)		
MAP3K11 (rs1784223)												
TT/TC	1717	1368	1		0.831	0.995	838	683	1.00		0.046	0.137
CC	239	187	0.98	(0.80, 1.20)			121	71	0.73	(0.53, 0.99)		
MAPK1 (rs11913721)												
AA	661	524	1		0.277	0.862	328	286	1.00		0.028	0.111
AC	984	750	0.97	(0.84, 1.13)			456	361	0.92	(0.75, 1.14)		
CC	295	268	1.16	(0.94, 1.42)			171	102	0.70	(0.52, 0.94)		
MAPK8 (10857561)[d]												
GG/GA	1753	1379	1		0.243	0.474	876	663	1		0.015	0.030
AA	203	176	1.14	(0.92, 1.41)			83	91	1.48	(1.08, 2.03)		
Raf1 (rs4684871)												
AA/AG	1623	1303	1		0.646	1.000	792	653	1		0.022	0.111
GG	333	252	0.96	(0.80, 1.15)			167	99	0.73	(0.56, 0.96)		

[a]OR and 95% CI were adjusted for age, study center, race and sex.
[b]Similar associations for *MAP3K9* rs11624934 (r^2 = 0.74 with rs11625206 and r^2 = 0.77 with rs11628333).
[c]Similar associations for *MAP3K9* rs8010714 (r^2 = 0.99).
[d]Similar associations for *MAPK8* rs10508901 (r^2 = 1).

SNP spectral decomposition method proposed by Nyholt (18) and modified by Li et al. (19) on the full sample of cases and controls. Adjustments were based on *P* values for 1-df Wald test statistics for main effects, tumor molecular phenotype and survival analysis. Adjustments for interactions were based on *P* values for 1-df likelihood-ratio tests. Adjusted *P* values of <0.10 were considered potentially important given the conservative nature of the Bonferroni correction. These associations are highlighted in the text and in bold font in the tables. Associations at this level are considered to guard against missing important associations that should be replicated in other studies. Additionally, given limited power to detect potentially important associations with recessive models, reporting associations at this level provides important information.

Results

Approximately, 90% of the population was non-Hispanic white. The majority of cases were male (56.0% of colon and 59.8% of rectal) and older than 60 years of age (73.3% of colon cancer cases and 58.8% of rectal study). Descriptive tables of the host population (Table I) and the candidate genes associated in subsequent analysis with an adjusted *P* value of <0.15 (Table II) are provided. All SNPs were in Hardy-Weinberg Equilibrium. A summary of all SNPs tested and their main effects for colon and rectal cancer can be found in Supplementary Table (available at *Carcinogenesis* Online).

Associations between candidate genes and colon and rectal cancer were modest (Table III). Two SNPs in *MAP2K1* (rs7181936 and rs8039880), two SNPs in *MAP3K3* (rs11658329 and rs3785574), one in *MAP3K7* (rs13208824), three in MAP3K9 (rs11625206, rs11628333 and rs11844774) and one in *MAP3K10* (rs1129156) were associated with colon cancer. All but four of these SNPs had adjusted *P* values of <0.10. The strongest association was observed for the GG genotype of *MAP2K1* rs8039880, where the risk estimate was 0.57

Table IV. Interaction between aspirin, smoking, estrogen status, BMI and MAPK genes

	Controls	Cases	OR[a]	(95% CI)	Controls	Cases	OR	(95% CI)	Int *P*	Holm *P*
	No recent aspirin/NSAID use				Recent aspirin NSAID use					
Colon										
MAP3K10 (rs892117)										
TT	301	266	1.00		210	145	0.79	(0.60, 1.04)	0.044	0.087
TC	573	520	1.05	(0.86, 1.29)	381	225	0.69	(0.54, 0.87)		
CC	262	267	1.19	(0.93, 1.51)	213	115	0.63	(0.47, 0.83)		
Rectal										
MAP3K1 (rs2548663)[b]										
AA	263	202	1.00		200	141	0.93	(0.70, 1.24)	0.006	0.026
AG/GG	258	275	1.37	(1.06, 1.76)	228	130	0.73	(0.55, 0.98)		
MAPK1 (rs2298432)										
CC	219	206	1.00		193	96	0.54	(0.39, 0.73)	0.002	0.010
CA	218	212	1.06	(0.81, 1.38)	188	127	0.74	(0.55, 0.99)		
AA	84	59	0.76	(0.52, 1.12)	47	48	1.11	(0.71, 1.74)		
MAPK12 (rs2272857)										
GG	308	262	1.00		227	162	0.85	(0.65, 1.11)	0.032	0.061
GA	176	178	1.18	(0.90, 1.54)	168	91	0.64	(0.47, 0.87)		
AA	31	33	1.22	(0.72, 2.05)	31	17	0.61	(0.33, 1.13)		
MAPK14 (rs851016)[c]										
AA	386	384	1.00		340	194	0.58	(0.46, 0.73)	0.001	0.008
AG	124	88	0.73	(0.54, 1.00)	82	71	0.88	(0.62, 1.25)		
GG	11	5	0.48	(0.17, 1.41)	6	6	1.03	(0.33, 3.24)		
	Non-smoker/non-recent smoker				Recent smoker					
Colon										
MAP3K11 (rs1784223)										
TT	690	555	1.00		165	124	0.91	(0.70, 1.19)	0.006	0.018
TC	713	536	0.94	(0.80, 1.10)	149	151	1.22	(0.95, 1.58)		
CC	206	143	0.86	(0.68, 1.10)	32	43	1.62	(1.01, 2.60)		
MAP3K11 (rs7116712)										
TT/TC	1333	1024	1.00		277	278	1.27	(1.05, 1.53)	0.025	0.051
CC	276	209	0.99	(0.81, 1.20)	69	40	0.74	(0.49, 1.10)		
Rectal										
DUSP1 (rs322351)										
CC	246	179	1.00		57	36	0.81	(0.51, 1.29)	0.014	0.025
CT	399	281	0.99	(0.77, 1.27)	70	74	1.44	(0.99, 2.12)		
TT	164	142	1.23	(0.91, 1.66)	23	38	2.38	(1.36, 4.14)		
DUSP1 (rs881150)										
TT	459	350	1.00		77	98	1.63	(1.17, 2.27)	0.041	0.041
TA	297	212	0.95	(0.76, 1.19)	64	43	0.87	(0.57, 1.31)		
AA	53	40	1.02	(0.66, 1.57)	9	7	0.98	(0.36, 2.68)		
MAP3K11 (rs1784223)										
TT	364	265	1.00		52	71	1.81	(1.22, 2.68)	0.026	0.078
TC/CC	445	337	1.05	(0.85, 1.30)	98	77	1.06	(0.76, 1.49)		
MAPK8 (rs4838590)[d]										
CC	275	180	1.00		42	56	1.99	(1.28, 3.11)	0.015	0.030
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)		
Raf1 (rs9809501)										
TT	669	474	1.00		118	130	1.51	(1.15, 2.00)	0.006	0.032
TG/GG	140	128	1.29	(0.99, 1.69)	32	18	0.77	(0.42, 1.38)		

(Table IV continued)

	Controls	Cases	OR[a]	(95% CI)	Controls	Cases	OR	(95% CI)	Int *P*	Holm *P*
	No recent estrogen exposure				Recent estrogen exposure					
Colon										
DUSP1 (rs881150)										
TT	316	243	1.00		181	119	0.71	(0.51, 0.97)	0.009	0.016
TA	174	172	1.33	(1.02, 1.75)	149	96	0.69	(0.49, 0.97)		
AA	33	34	1.35	(0.81, 2.25)	31	7	0.24	(0.10, 0.56)		
DUSP2 (rs1724120)										
GG/GA	433	351	1.00		277	183	0.66	(0.50, 0.87)	0.031	0.031
AA	90	98	1.31	(0.95, 1.80)	84	39	0.48	(0.31, 0.74)		

	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)	Controls	Cases	OR	(95% CI)	Int *P*	Holm *P*
	Normal (<25)				Overweight (25–29)				Obese (≥30)					
Colon														
DUSP4 (rs2341674)														
CC	512	348	1.00		541	438	1.19	(0.99, 1.44)	295	276	1.35	(1.09, 1.67)	0.020	0.081
CT	222	141	0.93	(0.72, 1.19)	233	174	1.07	(0.84, 1.36)	99	126	1.84	(1.37, 2.49)		
TT	24	15	0.92	(0.48, 1.79)	22	18	1.14	(0.60, 2.16)	4	13	5.06	(1.63, 15.69)		
MAP3K2 (rs3732209)														
TT	367	259	1.00		397	334	1.17	(0.94, 1.46)	218	194	1.24	(0.96, 1.60)	0.022	0.057
TC	317	202	0.91	(0.71, 1.15)	327	242	1.05	(0.83, 1.33)	148	176	1.67	(1.27, 2.18)		
CC	74	43	0.84	(0.55, 1.26)	71	54	1.09	(0.74, 1.61)	32	44	1.93	(1.19, 3.13)		
Rectal														
DUSP1 (rs881150)														
TT	159	154	1.00		230	190	0.83	(0.61, 1.12)	143	104	0.74	(0.52, 1.03)	0.0009	0.002
TA/AA	152	89	0.62	(0.44, 0.87)	178	112	0.64	(0.46, 0.89)	92	100	1.1	(0.76, 1.57)		
MAP3K1 (rs16886403)														
TT	253	174	1.00		346	242	0.99	(0.76, 1.29)	186	168	1.27	(0.95, 1.70)	0.019	0.074
TC/CC	58	69	1.71	(1.14, 2.55)	62	60	1.32	(0.87, 2.00)	49	36	1.03	(0.64, 1.65)		

[a]OR and 95% CIs were adjusted for age, study center, race and sex.
[b]Similar associations for *MAP3K1* rs702689 ($r^2 = 1$) and rs33323 ($r^2 = 0.74$).
[c]Similar associations for *MAPK14* rs851011 ($r^2 = 0.99$).
[d]Similar associations for *MAPK8* rs11101320 ($r^2 = 0.98$).

Table V. Associations between tumor molecular phenotype and MAP kinase genes and risk of colon cancer

	Controls	Cases	OR[a]	(95% CI)	Wald *P*[b]	Holm *P*
MAP3K7 (rs150117)	*KRAS* mutation					
AA	926	142	1.00		0.001	0.006
AT/TT	1030	206	1.32	(1.06, 1.63)		
MAPK3 (rs7698)						
CC	1675	284	1.00		0.016	0.016
CT/TT	274	64	1.34	(1.02, 1.76)		
MAPK1 (rs11913721)[c]	*TP53* mutation					
AA	661	159	1.00		0.021	0.085
AC/CC	1279	350	1.22	(1.01, 1.47)		
MAPK1 (rs8136867)						
AA	537	166	1.00		0.045	0.130
AG	1008	255	0.84	(0.69, 1.03)		
GG	411	95	0.73	(0.57, 0.95)		
MAPK14 (rs851006)	CIMP+					
GG/GA	1846	245	1.00		0.029	0.147
AA	110	27	1.81	(1.23, 2.65)		
MAPK14 (rs851016)[d]						
AA/AG	1921	259	1.00		0.003	0.018
GG	35	13	2.81	(1.61, 4.89)		
MAP3K11 (rs7116712)	MSI+					
TT	713	82	1.00		0.038	0.114
TC	898	78	0.76	(0.56, 1.03)		
CC	345	24	0.60	(0.39, 0.95)		
MAPK1 (rs9610375)[e]						
AA	559	72	1.00		0.002	0.006
AC/CC	1397	113	0.63	(0.47, 0.84)		
MAPK1 (rs8136867)						
AA	537	47	1.00		0.007	0.020
AG	1008	83	1.01	(0.71, 1.44)		
GG	411	55	1.62	(1.10, 2.38)		
MAP3K9 (rs11625206)[f]	CIMP+ and MSI+					
CC/CT	1777	86	1.00		0.009	0.109
TT	178	22	2.52	(1.55, 4.08)		
MAP3K9 (rs11628333)[f]						
TT/TC	1720	86	1.00		0.016	0.167
CC	235	22	1.83	(1.14, 2.96)		
MAP3K9 (rs11844774)[g]						
TT	626	50	1.00		0.029	0.246
TC/CC	1329	58	0.57	(0.39, 0.84)		

[a]OR and 95% CIs were adjusted for age, study center, race and sex.
[b]Wald *P* value is for significant difference in association between other tumor molecular phenotype.
[c]Similar associations for *MAPK1* rs9610375 ($r^2 = 0.82$).
[d]Similar associations for *MAPK14* rs851011 ($r^2 = 0.99$).
[e]Similar associations for *MAPK1* rs11913721 ($r^2 = 0.82$).
[f]Similar associations for *MAP3K9* rs11624934 ($r^2 = 0.74$ with rs11625206 and $r^2 = 0.77$ with rs11628333).
[g]Similar associations for *MAP3K9* rs8010714 ($r^2 = 0.99$).

with 95% CI of (0.38, 0.83). Five different genes and SNPs were associated with rectal cancer: *DUSP1* rs322351, *MAP3K11* rs1784223, *MAPK1* rs11913721, *MAPK8* rs10857561 and *Raf1* rs4684871. All of these SNPs had about 40–50% increased risk for the high-risk genotype.

We observed several interactions between MAPK genes and use of aspirin/NSAID, cigarette smoking, estrogen and BMI. Table IV shows those that had adjusted *P* values of <0.10; Supplementary Table (available at *Carcinogenesis* Online) shows those that had significant unadjusted *P* values but adjusted *P* values of 0.10 or greater. For both aspirin/NSAID and cigarette smoking, we observed more significant interactions for rectal cancer than for colon cancer; genes that interacted were different for the two disease sites. For colon cancer, taking aspirin/NSAID significantly reduced risk only among those with a variant allele of *MAP3K10* rs892117. For rectal cancer, aspirin/NSAID users had a greater reduced risk if they also had a variant allele of *MAP3K1* rs2548663 or *MAPK12* rs2272857, whereas those not taking aspirin/NSAID with this allele were at increased risk of rectal cancer. For *MAPK1* rs2298432 and *MAPK14* rs851016, using aspirin/NSAIDs was most protective among those with the wild-type genotype. Cigarette smoking had the greatest impact on risk of colon cancer among those with the CC genotype of *MAP3K11* rs1784223 and the TT/TC genotypes of *MAP3K11* rs7116712 based on adjusted *P* values. For rectal cancer being a smoker and having the TT genotype of *DUSP1* rs322351, the TT genotype of *DSUP1* rs881150, the TT genotype of *MAP3K1* rs17842231, the CC genotype of *MAPK8* rs4838590 and the TT genotype of *Raf1* rs9809501 significantly increased risk. Recent use of estrogen was most protective for colon cancer among those with the AA genotype of *DUSP1* rs881150 and the AA genotype of *DUSP2* rs1724120. Obesity had its greatest effect on colon cancer risk among those with the TT genotype of *DUSP4* rs2341674 and the CC genotype of *MAP3K2* rs3732209. Those with normal weight were at reduced risk of rectal cancer if they also had the TA/AA genotypes of *DUSP1* rs881150, whereas being at increased risk of rectal cancer if they had the TC/CC genotypes of *MAP3K1* rs16886403.

Several candidate genes showed unique associations with specific colon cancer molecular phenotypes (Table V). *MAP3K7* and *MAPK3* were uniquely associated with *KRAS*, *MAPK1* was associated with *TP53* mutations, *MAPK14* was associated with CIMP+ tumors, *MAP3K11* and *MAPK1* were associated with MSI and *MAP3K9* was associated uniquely with CIMP+ and MSI. It is interesting to note the *MAPK1* rs8136867 was inversely associated with *TP53* while increasing risk of an MSI+ tumor.

Table VI. Associations between tumor molecular phenotype and MAP kinase genes and risk of rectal cancer

	Controls	Cases	OR[a]	(95% CI)	Wald *P*[b]	Holm *P*
MAP3K10 (rs892117)		*KRAS2* mutation				
TT	230	29	1.00		0.001	0.002
TC	469	86	1.46	(0.96, 2.23)		
CC	260	58	1.81	(1.16, 2.83)		
MAP3K9 (rs11625206)						
CC/CT	852	164	1.00		0.017	0.200
TT	107	9	0.48	(0.24, 0.94)		
Raf1 (rs11711419)[c]						
AA	651	98	1.00		0.001	0.005
AT/TT	308	75	1.63	(1.21, 2.21)		
Raf1 (rs3729931)						
CC	388	54	1.00		0.001	0.006
CT/TT	571	119	1.49	(1.08, 2.07)		
Raf1 (rs4684871)						
AA	360	49	1.00		0.011	0.034
AG/GG	599	123	1.58	(1.13, 2.20)		
DUSP6 (rs770087)[d]		*TP53* mutation				
TT	602	193	1.00		0.036	0.037
TG/GG	357	84	0.75	(0.58, 0.98)		
MAP3K11 (rs1784223)						
TT	416	139	1.00		0.007	0.022
TC	422	116	0.81	(0.63, 1.05)		
CC	121	22	0.56	(0.35, 0.88)		
MAPK8 (rs10857561)[e]						
GG	448	116	1.00		0.018	0.032
GA	428	119	1.16	(0.89, 1.52)		
AA	83	42	1.87	(1.28, 2.72)		
MAPK8 (rs10857565)						
GG	593	154	1.00		0.039	0.039
GA	325	104	1.24	(0.95, 1.61)		
AA	41	18	1.77	(1.04, 2.99)		
MAPK8 (rs11101320)[f]						
GG	331	79	1.00		0.036	0.036
GA	456	135	1.27	(0.95, 1.69)		
AA	172	63	1.50	(1.06, 2.11)		
MAP3K7 (rs3799912)		CIMP+				
AA/AG	945	56	1.00		0.027	0.135
GG	14	3	3.57	(1.07,11.91)		
MAP3K9 (rs17766621)						
TT	430	33	1.00		0.008	0.093
TC	415	25	0.75	(0.45, 1.26)		
CC	114	1	0.11	(0.02, 0.78)		
MAP3K9 (rs4902854)						
CC	359	29	1.00		0.015	0.160
CT/TT	600	30	0.58	(0.35, 0.96)		
Raf1 (rs4684871)						
AA	360	29	1.00		0.031	0.156
AG/GG	599	30	0.58	(0.35, 0.97)		

[a]OR and 95% CIs adjusted for age, study center, race and sex.
[b]Wald *P* value is for significant difference between associations with other tumor molecular phenotypes.
[c]Similar associations for *Raf1* rs11923427 ($r^2 = 0.84$).
[d]Similar associations for *DUSP6* rs10744 ($r^2 = 1$).
[e]Similar associations for *MAPK8* rs10508901 ($r^2 = 1$).
[f]Similar associations for *MAPK8* rs4838590 ($r^2 = 0.98$).

MAP3K10, *MAP3K9* and three *Raf1* SNPs were associated with *KRAS*-mutated rectal tumors; *DUSP6*, *MAP3K11* and three *MAPK8* SNPs were associated with *TP53*-mutated rectal tumors (Table VI). *MAP3K7*, *MAP3K9* and *Raf1* were associated with CIMP+ rectal tumors. Although *MAP3K9* was associated with CIMP+ tumors for both colon and rectal cancer, the SNPs associated were different for the two cancer sites.

Several genes were associated with survival for both colon and rectal cancer (Table VII). Variant alleles in *DUSP2* rs1724120 and *MAP3K9* rs17766621 reduced risk of dying after diagnosis with colon cancer, whereas *MAP3K1* rs33323, *MAP3K10* rs1129156, *MAP3K11* rs11227234 and rs1151488 increased the hazard of dying after diagnosis with colon cancer. The hazard of dying after diagnosis with rectal cancer increased among those with the variant alleles of *MAP2K1* rs17259670 and rs8039880 and *MAPK14* rs10807156. The homozygote variant genotype of *MAP3K9* rs17766621 and *MAP3K11* rs7116712 reduced risk of dying after diagnosis with rectal cancer. Four SNPs in *Raf1* were associated with survival after diagnosis with rectal cancer. The variant allele of *MAP3K9* rs17766621 reduced the risk of dying for both colon and rectal cancer.

Discussion

Our findings illustrate the multifaceted role of MAPK in colon and rectal cancer. Several genes representing the three most studied MAPK-signaling pathways were associated with colon and rectal

Table VII. Associations between MAPK genes and survival after diagnosis with colon or rectal cancer

	Death/person years	HRR[a]	(95% CI)	Wald *P*	Holm *P*
Colon					
DUSP2 (rs1724120)					
GG/GA	248/6338	1.00		0.024	0.024
AA	61/1810	0.72	(0.54, 0.96)		
MAP3K1 (rs33323)[b]					
GG/GC	247/6870	1.00		0.033	0.131
CC	62/1278	1.37	(1.03, 1.82)		
MAP3K9 (rs17766621)					
TT	147/3465	1.00		0.040	0.466
TC/CC	162/4683	0.79	(0.63, 0.99)		
MAP3K10 (rs1129156)					
CC	170/4571	1.00		0.005	0.010
CT/TT	138/3574	1.40	(1.10, 1.76)		
MAP3K11 (rs11227234)					
GG/GT	282/7642	1.00		0.006	0.018
TT	27/506	1.76	(1.18, 2.62)		
MAP3K11 (rs1151488)					
AA	138/4035	1.00		0.042	0.083
AG/GG	171/4113	1.27	(1.01, 1.60)		
Rectal					
MAP2K1 (rs17259670)					
AA	140/3657	1.00		0.048	0.239
AG/GG	31/632	1.49	(1.00, 2.22)		
MAP2K1 (rs8039880)					
AA/AG	160/4086	1.00		0.004	0.025
GG	11/203	2.53	(1.34, 4.79)		
MAP3K9 (rs17766621)					
TT/TC	160/3722	1.00		0.049	0.567
CC	11/568	0.53	(0.28, 1.00)		
MAP3K11 (rs7116712)					
TT	73/1599	1.00		0.037	0.110
TC	80/1936	0.88	(0.64, 1.22)		
CC	18/748	0.56	(0.33, 0.94)		
MAPK14 (rs10807156)					
TT/TA	153/4044	1.00		0.040	0.279
AA	17/240	1.73	(1.03, 2.91)		
Raf1 (rs11923427)[c]					
CC	135/3049	1.00		0.006	0.031
CG/GG	36/1225	0.59	(0.40, 0.86)		
Raf1 (rs4684871)					
AA	67/1533	1.00		0.028	0.085
AG	85/2176	0.80	(0.57, 1.12)		
GG	19/573	0.56	(0.33, 0.96)		
Raf1 (rs904453)					
CC	41/1184	1.00		0.014	0.058
CA	81/2159	1.19	(0.81, 1.75)		
AA	49/947	1.73	(1.12, 2.67)		
Raf1 (rs9809501)					
TT	145/3443	1.00		0.033	0.085
TG/GG	26/846	0.62	(0.40, 0.96)		

[a]Adjusted for age, study center, race, sex, AJCC stage and tumor markers.
[b]Similar associations for *MAP3K1* rs2548663 ($r^2 = 0.74$) and rs702689 ($r^2 = 0.74$).
[c]Similar associations for *RAF1* rs11711419 ($r^2 = 0.84$).

cancer overall, and others had unique associations with specific tumor molecular phenotype, influenced survival and interacted with lifestyle factors that are associated with inflammation, oxidative stress and hormones, that is, aspirin, smoking, estrogen status and BMI.

MAPKs mediate intracellular signaling and are involved in diverse cellular processes that include cell proliferation and differentiation and apoptosis. As such, they are implicated in cancer development and progression (20). MAPK-signaling systems are activated by extracellular stimuli that results in intracellular response. They provide a link between transmembrane signaling and changes in transcription in response to various environmental signals such as cytokines, growth factors, oxidative stress and inflammation. The three major categories of MAPK that have been most thoroughly studied are the stress-activated protein kinase 1 (*JNK* or *SAPK1)*, stress-activated protein kinase 2 (*p38* or *SAPK2*) and the extracellular signal-regulated protein kinases (*ERK1*/2) (20,21). JNK is generally associated with apoptosis induction; ERK1 and 2 are generally associated with mitogenesis; *p38* has been described as being involved in both (2).

Few studies have evaluated the genetic variation in MAPK and cancer. A CRC genome-wide association study conducted in Germany identified MAPK-signaling pathway as an important pathway in colon cancer (6). Seven SNPs in the MAPK pathway were identified as being related to colon cancer with increasing risk being associated with increasing number of risk alleles in the seven genes involved in MAPK signaling. Although the MAPK-signaling pathway was implicated in CRC in the German study, none of the SNPs associated with colon or rectal cancer identified in this study was among the top hits in that study. In this study, we evaluated colon and rectal

cancer separately, whereas in that study, both were combined. Our results suggest differences in effect for colon and rectal cancer and support the previous observation that cell type or tissue may influence the cellular response. A study by Hardwick *et al.* also has linked the MAPK-signaling pathway to colon cancer (22). They observed that both p38 and JNK were highly expressed in colonic adenomatous polyps. Others also have shown that ERK and JNK are upregulated in colorectal carcinomas (23). Our results suggest that genetic variation in the MAPK-signaling pathway influence both colon and rectal cancer risk. DUSPs, which attenuate the effect of MAPK (24), also were associated with colon and rectal cancer. Associations were observed for colon cancer for several genes that relate to both the JNK- and p38-signaling pathways. Our data suggest that the ERK-signaling pathway is more associated with rectal cancer given the number of genes in this pathway associated with rectal cancer but not colon cancer.

MAPK are activated by external signaling, thus we evaluated interaction between genetic variants in the signaling pathway and environmental and lifestyle factors that are related to oxidative stress, inflammation and growth hormones. JNK and p38 pathways are activated by pro-inflammatory cytokines and oxidative stress. Reactive oxygen species also have been shown to activate JNK. JNK and ERK have been shown to be modulated by obesity and insulin resistance. In our data, aspirin/NSAID use, which may indicate level of inflammation, interacted with genetic variants in the JNK-signaling pathway for both colon and rectal cancer; however, ERK-related genes also interacted with aspirin/NSAID use for rectal cancer. Cigarette smoking, which can influence levels of oxidative stress, interacted with JNK and p38 pathways for both colon and rectal cancer. Variants in the JNK and ERK pathways interacted with BMI. DUSP, which would influence multiple signaling pathways, interacted with smoking for rectal cancer and with BMI for both colon and rectal cancer.

We identified unique associations with tumor molecular phenotype. The *Ras* family has been associated with ERK and thought to play a major role in transmission of extracellular signals into cells (2). Both JNK and ERK signaling has been associated with *TP53* (25,26). Additionally, CIMP, MSI and *TP53* have been associated with inflammation-related pathways and may, therefore, be influenced by signaling pathways that depend on inflammation as the stimulus for activation. *KRAS*-mutated tumors appeared to be associated with JNK and ERK-related variants, whereas *TP53* was associated with ERK2 for colon cancer but with JNK and p38 for rectal cancer. CIMP+ tumors were related to genetic variation in all three signaling pathways.

Given the MAPK-signaling pathways are associated with apoptosis and tumor growth, it is reasonable to evaluate the influence of genetic variants in this pathway on survival after diagnosis with cancer. We observed several genetic variants associated with survival. Genetic variation in the JNK pathway, which is associated with apoptosis induction, seemed to have the greatest influence on survival. For rectal cancer, *Raf1* also influenced survival. Although studies have shown that various MAPK pathways influence cell proliferation differently, we are limited in our understanding of genetic variation on cellular activity, given limited functionality work on these genes. Thus, although we observe that a given gene may be associated with a greater or lesser hazard of dying, we do not know how the genotype influences protein expression that is related to cellular activity. Work is needed to understand the functionality of these genes.

The study was hypothesis-driven, with a large and extensive data set that includes information on genetic, diet and lifestyle data, our ability to examine colon and rectal cancer separately, tumor molecular phenotype and survival. We view this as a major strength of this study. Although we believe that the data we present are both thorough and informative, we acknowledge that limitations exist. We selected tagSNPs to examine genetic variation across the gene, however, we could have missed important functional SNPs. Likewise, because we focused on SNPs that had a MAF of >0.10 so that we would have sufficient power to look at interactions, we could have missed associations with rarer genotypes. Also, although we have detected associations with our tagSNPs, we have minimal information on the functionality of the SNPs evaluated. Additional lab-based experiments are needed to determine functionality. The genes we selected were based on the literature at the time the platform was developed; however, other MAP kinase genes were not included that may be associated with colon and rectal cancer. Additionally, we have limited power to evaluate some molecular phenotype and colon and rectal cancer, so important associations could have been missed especially when evaluating associations with CIMP and MSI. Because of this, we present associations that were significant at the 0.05 level prior to adjustment with the adjusted *P* value for multiple comparisons. Through our analysis, we have made many comparisons. Although we have provided adjusted *P* values to take into account multiple comparisons, chance findings may exist and, therefore, replication of these findings is critical. A hazard of multiple testing adjustments is the increased likelihood of rejecting a finding that is true. Thus, we believe that adjusted *P* values greater than 0.05 are important especially for interactions and merit replication in other large sample sets to validate these findings. Evaluation of the number of SNPs associated may increase the odds that a gene is associated with CRC.

In summary, several genes involved in MAPK signaling appear to be associated with colon and rectal cancer. Some associations were dependent on use of aspirin/NSAIDs, cigarette smoking, estrogen exposure and level of BMI. Other genes were uniquely associated with various molecular phenotypes, further suggesting signaling pathways have distinct molecular targets. MAPK variants also appeared to influence survival. Additional work is needed to verify these associations, which could in part be from chance. Assessment of functionality of these genes would provide additional support for detected associations.

Supplementary material

Supplementary Table can be found at http://carcin.oxfordjournals.org/

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