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Genetic variation in *RPS6KA1*, *RPS6KA2*, *RPS6KB1*, *RPS6KB2*, and *PDK1* and risk of colon or rectal cancer

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Abstract

RPS6KA1, RPS6KA2, RPS6KB1, RPS6KB2, and PDK1 are involved in several pathways central to the carcinogenic process, including regulation of cell growth, insulin, and inflammation. We evaluated genetic variation in their candidate genes to obtain a better understanding of their association with colon and rectal cancer. We used data from two population-based case-control studies of colon (n=1574 cases, 1940 controls) and rectal (n=791 cases, 999 controls) cancer. We observed genetic variation in *RPS6KA1*, *RPS6KA2*, and *RPS6KB2* were associated with risk of developing colon cancer while only genetic variation in *RPS6KA2* was associated with altering risk of rectal cancer. These genes also interacted significantly with other genes operating in similar mechanisms, including *Akt1*, *FRAP1*, *NFκB1*, and *PIK3CA*. Assessment of tumor markers indicated that these genes and this pathway may importantly contributed to CIMP+ tumors and tumors with *KRAS2* mutations. Our findings implicate these candidate genes in the etiology of colon and rectal cancer and provide information on how these genes operate with other genes in the pathway. Our data further suggest that this pathway may lead to CIMP+ and *KRAS2*-mutated tumors.

Keywords

Colon cancer; rectal cancer; S6K; RSK; PDK1; Akt; CIMP+; *KRAS2*

The ribosomal protein S6 kinase (RPS6K) family is involved in numerous pathways, many of which are central to the carcinogenic process. The *RPS6K* alpha genes, also known as *RPS6KA1*, *RSK1*, or p90-S6K1 and *RPS6KA2*, *RSK2*, or p90-S6K2, are mitogen-activating protein kinases. *RSK1* has been identified as a key component in the regulation of IκBβ and NFκB[1], factors associated with gut inflammation and apoptosis[2,3], and it is weakly activated by insulin [4]. *RPS6KB1* (i.e. S6K1) and B2 (i.e. S6K2) also known as *p70-beta*, or *p54*, share 80% homology and many of the same functions. Studies suggests that *RPS6KB1* and *RPS6KB2* are involved in cell growth and regulation given their activation by growth factors such as EGF, PDGF, and insulin and their regulation by mammalian Target of Rapamycin (mTOR). They are keenly involved in regulation of insulin. Both *RPS6KS* and *RPS6KB* proteins are members of the AGC protein kinase family and require 3-phosphoinositide-dependent protein kinase-1 (PDK1) phosphorylation for

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activation [5,6]. PDK1 mediates the cellular influence of growth factors and insulin by activating both RSK and S6K and is essential for activation of PKB/Akt [5]

Studies have not examined genetic variation in the *RPS6KA1*, *RPS6KA2*, *RPS6KB1*, *RPS6KB2*, or the *PDK1* genes and risk of colon or rectal cancer. Given the biological role of these genes in the regulation of cell growth and inflammation, it is reasonable to hypothesize that genetic variation of these genes may influence risk of colon and rectal cancer, either independently or in combination with other genes and lifestyle factors. In this study we evaluate whether genetic variation in these genes alters susceptibility to colon or rectal cancer. Data come from two large population-based studies, one of colon cancer and one of rectal cancer. We evaluate potential interaction between SNPs within and between genes as well as with recent use of aspirin/NSAIDs (an indicator of inflammation given their anti-inflammatory properties), BMI (an indicator of insulin), and estrogen status (an indicator of estrogen). Because of the role of these genes with other genes, such as *FRAP1* (mTOR), *PIK3CA*, *Akt1*, and *NFκB1*, we also evaluate interaction with these genes. In an effort to more comprehensively understand how *RPS6KA1*, *RPS6KA2*, *RPS6KB1*, *PRK3CA*, and *PDK1* are associated with colon and rectal cancer we look at genetic variation in these genes with specific tumor markers to gain insight into disease pathways.

Methods

Data for the study come from two case-control studies conducted in Utah, the Northern California Kaiser Permanente Medical Care Program (KPMCP), and the Twin Cities Metropolitan area of Minnesota (colon cancer study only). Eligibility criteria included being between 30 and 79 years of age at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of colorectal cancer, and no known (as indicated on the pathology report or by self-report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease. Controls were frequency matched to cases by sex and five-year age groups. Controls were randomly selected from membership lists (KPMCP) or from driver's license list (Minnesota). In Utah, controls 65 or older were randomly selected from lists provided by the Centers for Medicare and Medicaid Services and controls younger than 65 were randomly selected from driver's license lists. Study eligibility and recruitment details of the study have been published previously [6,7]. Cooperation rates were 83% at KPMCP, 76% at Utah, and 67% Minnesota (colon cases); 73% at KPMCP, 69% at Utah, and 53% at Minnesota (colon controls); 75.4% at KPMCP and 69.7% at Utah (rectal cases); 69.9% at KPMCP and 67.2% at Utah (rectal controls).

The current analysis is restricted to subjects who provided a blood sample. The colon cancer study population consists of non-Hispanic white cases ($n = 1444$) and controls ($n = 1841$), Hispanic or American Indian cases ($n = 60$) and controls ($n = 75$), and African American cases ($n = 70$) and controls ($n = 54$). The rectal cancer study population consists of non-Hispanic white cases ($n = 657$) and controls ($n = 856$), Hispanic or American Indian cases ($n = 63$) and controls ($n = 69$), African American cases ($n = 31$) and controls ($n = 44$), and Asian cases ($n = 40$) and controls ($n = 30$). Race and ethnicity were self-reported at the time of interview.

Trained and certified interviewers collected diet and lifestyle data as previously described [8,9] using the same data questionnaire and study procedures for both the colon and rectal studies. The referent year for the study was the calendar year approximately two years prior to date of diagnosis (cases) or selection (controls). Information was collected on demographic factors such as age, sex, and study center, and on exposures including diet, physical activity, aspirin and non-steroidal drug use, body size, and other lifestyle factors including medical, family, and reproductive history.

DNA was extracted from blood drawn from study participants. Tagging SNPs were characterized as part of a customized Illumina bead array platform. TagSNPs were identified using HapMap and the Illumina Platform tagSNP database. TagSNPs were selected for *RPS6KA1*, *PDK1*, *RPS6KB1*, and *RPS6KB2* using the following parameters: an $r^2=0.9$ defined LD blocks using a Caucasian LD map, minor allele frequency or MAF>0.1, and range= -1500 bps from the initiation codon to +1500 bps from the termination codon. The *RPS6KA2* gene parameters were changed to an $r^2=0.5$ given the gene size. We successfully tested 12 tagSNPs for *RPS6KA1*, 72 for *RPS6KA2*, 6 for *RPS6KB1*, 2 for *RPS6KB2*, and 3 for *PDK1*. Individual tagSNPs were assessed to determine if they were in Hardy-Weinberg equilibrium (HWE) in non-Hispanic white controls, constituting approximately 91% of the study controls, and were not carried forward if this criterion was not met after controlling the false discovery rate [10]. The sample success rate was 97.55%, the locus success rate was 89.91%; the genotype call rate was 99.85%. There was 100% reproducibility in quality control samples. Appendix 1 shows a list of all tagSNPs assessed. Table 1 summarizes those tagSNPs either associated independently or in combination with other candidate genes.

Tumor Marker Data

We have previously evaluated tumors for CpG island methylator phenotype (CIMP), microsatellite instability (MSI), *TP53* mutations, and *KRAS2* mutations [11–14] and were therefore able to evaluate with genes assessed. Details for methods used to evaluate these epigenetic and genetic changes have been described in previous publications [11–14].

Statistical Analysis

Statistical analysis involved several steps and focused on testing the following hypotheses: SNPs within the candidate genes are associated with colon or rectal cancer; SNPs within candidate genes act jointly to alter risk of colon and rectal cancer; factors associated with insulin and inflammation that may influence individual susceptibility to genetic variation in these genes; and genetic variation in the candidate genes may influence specific disease pathways as indicated by epigenetic and genetic changes in tumors. Recent use (within two years of diagnosis) of aspirin/NSAIDs was used as an indicator of inflammation state and BMI was used as a crude indicator of insulin sensitivity. Initial weeding of tagSNPs was done by dropping one of the two SNPs if the r -squared measure of LD between the two was greater than or equal to 0.8, preferentially selecting the SNP with the lower proportion missing.

SAS 9.2 (Cary, NC) was used to perform all of the statistical analyses. SNPs from each gene were evaluated for their importance using step-wise logistic regression to determine which tagSNPs independently entered the step-wise logistic regression model and remained in the model as significant except when use of hapConstructor is noted below. Multiplicative interaction models that included multiple tagSNPs within a gene as well as tagSNPs from other genes in the pathway were assessed both at an individual tagSNP and haplotype level.

Odds ratios for SNPs adjusted for age at diagnosis or selection, race/ethnicity, sex, and study site were calculated based on a specific inheritance model that predicted the best fit. The MAX test was used to assess whether there was any relationship (recessive, dominant, or unrestricted) between colon or rectal cancer and the individual tagSNPs. Additionally, haplotypes were constructed for those tagSNPs within genes that remained significant in the stepwise logistic regression models. Haplotypes were constructed based on tagSNPs that were important for each phenotype using the EM algorithm. Each haplotype was weighted within the logistic regression model based upon the haplotype probability estimate and compared to all other haplotypes for a given set of tagSNPs. Adjustment of BMI, cigarette smoking, dietary energy, fiber, and calcium, long-term vigorous physical activity, regular

use of aspirin/NSAIDs within the past two years, and family history of colorectal cancer in first-degree relatives did not appreciably alter the risk estimates, therefore data are presented for the minimally adjusted model.

We used the data mining technique implemented in hapConstructor (a module of Genie 2.6.2 software; <http://www-genepi.med.utah.edu/Genie/index.html>) as a tool to evaluate combinations of SNPs across selected genes. A user-specified significance threshold of 0.01 was selected at two-locus step and p of 0.001 at the three locus step to take into account the number of comparisons being made for five genes. This level of stringency for significance in hapConstructor has been used previously in similar types of analyses [15]. The multi-locus genotype results from hapConstructor were then assessed using stepwise multiplicative interaction logistic regression models. We evaluated interaction between tagSNPs using a goodness of fit test that compared the maximum likelihood ratios of a model with and without an interaction term using Chi-squared test with one degree of freedom.

The phenotypic characteristics of the tumors were defined by the specific alterations detected and described as any *TP53* mutation, any *KRAS2* mutation, MSI+, CIMP+ defined as at least two of five markers methylated, or *KRAS2* mutated plus CIMP+, or CIMP+ plus MSI+ to describe the phenotypic characteristics of the tumor. As the proportion of MSI+ tumors in the rectal cases was <3% [16], there was insufficient power to examine these tumor markers with genotype data. Population-based controls were used to assess associations for the population overall when examining multiple outcomes defined by tumor status. In order to compare specific types of mutations to controls while adjusting for the tumor mutations simultaneously in cases, a generalized estimating equation (GEE) with a multinomial outcome was used [17], because case subjects could contribute to multiple outcome observations depending on the number of tumor alterations or mutations (*TP53*, *KRAS2*, CIMP+, and additionally for colon cases, MSI+ and *BRAF* V600E) an individual had [18]. The GEE accounts for correlated outcomes introduced by including subjects multiple times and was implemented in SAS using the GENMOD procedure as described by Kuss and McLerran [19]. Since no significant associations were detected with survival after adjusting for stage and tumor markers, data are not presented for survival.

Results

An association was detected between the rs12025634 in the *RPS6KA1* gene and colon cancer (Table 2). Additionally, three SNPs in the *RPS6KA2* gene, rs16898963, rs2072638, and rs9459678, were independently associated with colon cancer. A recessive model was the most appropriate model for each of these SNP. A haplotype of these three tagSNPs (data not shown in table) showed a significant 20% increased risk (95% CI 1.06, 1.36) for one copy of the A-C-T (*RPS6KA2* rs16898963, rs1072638, and rs9459678); this haplotype was present in 22% of the population.

Eight SNPs in *RPS6KA2* were associated significantly with rectal cancer (Table 3). The association with rs9295361 was confounded by rs6911624 and the association with rs6456123 was confounded by rs2072638. The magnitude of the associations ranged from having a protective effect for rs6911624 (OR 0.58, 95% CI 0.32, 1.04) to a twofold increased risk for rs9295361 (OR 2.00, 95% CI 1.24, 3.23 for the recessive model). A haplotype analysis of the important tagSNPs showed three statistically significant haplotypes for the *RPS6KA2* rs10946164, rs2072638 (T>C), rs2345067 (T>C), rs6911624, rs7745781, rs9295361, rs9347128. The T-C-C-G-C-T-A haplotype had a reduced risk of 0.83 (95% CI 0.69,1.00) per copy; the C-C-C-G-T-C-G haplotype was associated with an OR of 1.70 (95% CI 1.28,2.25); and the C-G-C-G-T-C-A haplotype was associated with an

OR of 1.56 (95% CI 1.13,2.15). These haplotypes were present in 8%, 3%, and 2% of the population, respectively.

Evaluation of combined genotypes showed several potentially important interactions between genotypes in the *RPS6KA1* and *RPS6KA2* SNPs (Table 4). For colon cancer, *RPS6KA2* rs3799620 interacted with rs9459678 (interacting p value 0.01) and rs16898963 (interaction p<0.01); rs1894660 interacted with rs6926393 (interaction p<0.01); rs2049956 interacted with rs6926393 (interaction p 0.02); and rs2072638 interacted with rs9459678 (interaction p=0.04) (data not shown in table). *RPS6KA2* rs2071941 interacted significantly with rs9347128 in rectal cancer (data not shown in table). *RPS6KB1* rs180531 interacted with Akt1 rs2494738; *RPS6KA2* rs3799620 interacted significantly with *RPS6KA1* rs12025634; and *RPS6KA2* rs2072638 interacted significantly with *FRAP1* rs1057079 among colon cancer cases and controls (Table 4). For rectal cancer cases and controls, *RPS6KB1* rs180519 and *PIK3CA* rs7640662 interacted significantly, as did *RPS6KB1* rs180515 and *RPKAG2* rs1104897, and the combination of *RPS6KB2* rs917570 and *PIK3CA* rs76400662.

Statistically significant interactions were observed between *RPS6KA1* rs12025634 and BMI and between *RPS6KA1* rs12025634 and recent estrogen exposure for colon cancer (Table 5). Having the TT genotype of rs12025634 resulted in over a six-fold increase risk of colon cancer among those with a BMI of 30 or more. Those without recent estrogen exposure were also at greater risk of colon cancer if they had the TT genotype of *RPS6KA1* rs12025634. For rectal cancer, recent aspirin/NSAID use was most protective among those who had the TT genotype of *RPS6KA2* rs2072638.

Assessment of those SNPs that were important from stepwise regression on various tumor phenotypes showed several interesting trends (Table 6). CIMP+ tumors were associated with *RPS6KA2* variants for both colon and rectal tumors. Similarly *RPS6KA2* was associated with *KRAS2*-mutated tumors for both colon and rectal cancer. Additionally, *RPS6KA1* was associated with *KRAS2*-mutated colon tumors and with *TP53* mutated colon tumors but not with rectal tumor mutations.

Discussion

RPS6KA1, *RPS6KA2*, *RPS6KB1*, *RPS6KB2*, and *PDK1* are part of a protein kinase family involved in the regulation of cellular growth, insulin, and cellular energy [20]. For both colon and rectal cancer, we observed several variants of these genes that influenced risk of developing the disease. For colon cancer, *RPS6KA1*, *RPS6KA2*, and *RPS6KB2* variants were associated with increased risk of the disease; for rectal cancer the risk was mainly associated in variants of *RPS6KA2*. Variants appeared to be mainly associated with a CIMP+ and *KRAS2*-mutated tumors.

Our data suggest that these genes are involved in pathways that include insulin, estrogen, and inflammation as would be hypothesized given what we currently know about their function. *RPS6KA1* is a component of the insulin signal transduction pathway, and may be a key element in regulating insulin resistance [21]; *RPS6KB* also has a role in growth factor regulation [22]. *PDK1* is needed to activate both *RPS6KB* and *S6K*, *RPS6KA* and thereby mediating the effect of insulin and growth factors [5,20]. NFκB plays a critical role in the regulation of inflammation and data have shown that *RPS6KB* is involved in a signaling pathway that involves angiotensin II activation of NFκB [23]. Estrogen also is involved in inflammation-related processes [24]. We observed statistically significant interactions with *RPS6KA1* and BMI and estrogen for colon cancer and *PDK1* and aspirin for both colon and rectal cancer. *RPS6KB2* interacted significantly with estrogen among those with rectal

cancer. BMI is associated with inflammation, estrogen, and insulin and thus the interaction supports the hypothesized mechanisms. Recent use of aspirin/NSAID may influence the inflammatory state of the gut and our data suggest that genetic factors with an inflammation-related mechanism are associated with altered risk of both colon and rectal cancer.

The candidate genes examined in this paper are associated with the PI3K/Akt/FRAP1 (mTOR) pathway [25,26]. mTOR plays a role in cellular proliferation as a nutrient sensor and controls RPS6K1; RPS6K2 is regulated by mTor and PI3K [27]. RPS6KA is involved in several stages of translational control and is a mediator in the PI3K/Akt/mTor pathway [22]. PDK1 is an important regulator of Akt1 [20]. Our data support the interaction of these pathways in that genetic variants of *RPS6KA1* interacted with variants of *Akt1* and *RPS6KB1* interacted with *mTOR* or *FRAP1* among colon cancer cases. For rectal cancer both *RPS6KB1* and *RPS6KB2* interacted with variants in *PIK3CA*. These results suggest that genetic variation within the pathway influences other genes within the pathway.

Our data suggest that these genes are involved in both a CIMP+ and *KRAS2* pathway. Data have shown that *KRAS2* is influenced by both the RPS6KB and the RPS6KA [28,29]. To our knowledge, no one has reported an association between genetic variation in *RPS6KA1* or *RPS6KA2* and CIMP+ colon or rectal tumors. We have previously reported an association between CIMP and other variants in insulin-related genes [30]. Our current data reinforce the hypothesis that *RPS6KA* is associated with CIMP+ and *KRAS2*-mutated tumors. It also illustrates the need to evaluate subsets of the data by tumor phenotype. Patterns of association with tumor markers provide a better understanding of mechanisms involved in the carcinogenic process.

We believe that the data presented here provide new insights into the etiology of colon and rectal cancers; however there also are study limitations that need to be recognized. A tagSNP approach was used to characterize the genetic variation in our candidate genes. Although we hypothesized that these genes would be important and that they would have interactions with the specific factors described, we did not have the necessary information to hypothesize that any specific SNP would be associated. We view the data as an indication of variation in the gene and not that any one SNP is causal. Thus, to determine a causal variant and impact on disease pathways, further work, including work to determine functionality of individual SNPs is needed. Likewise, while we hypothesize that use of aspirin/NSAIDs may indicate a lower level of gut inflammation, use of anti-inflammatory drugs could also serve as an anti-platelet agent. We have taken full advantage of our unique dataset and have evaluated these candidate genes with specific tumor markers to obtain insight into disease pathways. Our approach has lead to many comparisons and therefore replication of these associations in other large studies is needed.

There is a biological basis for our hypothesis that genetic variation in *RPS6KA1*, *RPS6KA2*, *RPS6KB1*, and *RPS6KB2*, and *PDK1* are associated with colon and rectal cancer. We have presented data that support the hypothesis that genetic variation in these genes contributes to risk of colon and rectal cancer. Our data further support that they are most important for a CIMP+ and *KRAS2* pathway and may further interact with genetic and lifestyle factors. Replication of these findings is needed.

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Table 1

Summary of key tagSNPs in candidate pathway

Symbol	Alias	Chromosome	SNP	MAF	Major/Minor Allele	FDR HWE Probability
<i>RPS6KA1</i>	<i>RSK1</i> <i>S6K-alpha1</i>	1p	rs12025634	0.14	C/T	0.39
<i>RPS6KA2</i>	<i>RSK</i> <i>RSK3</i> <i>S6K-alpha2</i> <i>p90-RSK3</i>	6q27	rs16898963 rs2071941 rs3799620 rs2345067 rs9295361 rs9459678 rs10946164 rs9347128 rs6911624 rs7745781 rs180519	0.12 0.14 0.37 0.44 0.21 0.34 0.42 0.41 0.19 0.13 0.45	A/C C/T A/G T/C C/A T/C C/T C/G G/A A/G G/A	0.21 0.93 0.53 0.64 0.91 0.8 0.84 0.89 0.97 0.9 0.61
<i>RPS6KB1</i>	<i>S6K1</i> p70-alpha p70(S6K) Alpha	17q23.1				
<i>RPS6KB2</i>	<i>S6K2</i> p70-beta p70(S6K) Beta	11q13.2	rs917570	0.44	C/G	0.89

Table 2
Associations between colon cancer and *RPS6KA1*, *RPS6KA2*, and *RPS6KB2*

	Colon			
	Controls		Cases	
	N	N	OR ^I	(95% CI)
<i>RPS6KA1</i> rs12025634(C>T)				
CC/CT	1928	1518	1.00	
TT	31	41	1.73	(1.08, 2.78)
P Max	0.07			
<i>RPS6KA2</i> rs9459678(T>C)				
TT/TC	1745	1345	1.00	
CC	214	213	1.25	(1.01, 1.53)
P Max	0.03			
<i>RPS6KA2</i> rs16898963(A>C)				
AA/AC	1937	1522	1.00	
CC	19	32	2.17	(1.22, 3.85)
P Max	0.02			
<i>RPS6KA2</i> rs2072638(T>C)				
TT	965	700	1.00	
TC/CC	993	856	1.21	(1.06, 1.38)
P max	0.03			
<i>RPS6KB2</i> (rs917570 C>G)				
CC	653	604	1.00	
CG/GG	1281	951	0.82	(0.71, 0.94)
P max	0.003			

^I Adjusted for age, center, race/ethnicity, and sex.

Table 3

Associations between *RPS6KA2* and rectal cancer

	Rectal			
	Controls	Cases	OR ^a	(95% CI)
<i>RPS6KA2</i> rs10946164(C>T)	N	N		
CC	312	292	1.00	
CT/TT	647	462	0.78	(0.63, 0.95)
P max	0.02			
<i>RPS6KA2</i> rs9347128(C>G)				
CC/CG	791	585	1.00	
GG	168	169	1.34	(1.05, 1.71)
P max	0.03			
<i>RPS6KA2</i> rs9295361(C>A)				
CC/CA	920	710	1.00	
AA	39	44	2.00	(1.24, 3.23)
p max	0.21			
<i>RPS6KA2</i> rs6911624(G>A)				
GG	614	530	1.00	
GA	308	206	0.77	(0.62, 0.95)
AA	37	18	0.58	(0.32, 1.04)
p max	0.01			
<i>RPS6KA2</i> rs2345067(T>C)				
TT	267	235	1.00	
TC	463	358	0.85	(0.68, 1.06)
CC	229	161	0.73	(0.56, 0.97)
p max	0.22			
<i>RPS6KA2</i> rs2072638(T>C)				
TT/TC	872	643	1.00	
CC	79	102	1.79	(1.31, 2.45)
p max	<0.01			

	Rectal			
	Controls		Cases	
	N	N	OR ^I	(95% CI)
<i>RPS6KA2</i> rs7745781 (A>G)				
AA	730	520	1.00	
AG/GG	229	234	1.40	(1.13, 1.74)

^I Adjusted for age, center, race/ethnicity, and sex.

Table 4

Interaction between genes in candidate pathway

Colon Cancer	Controls			Cases			Controls			Cases		
	N	N	OR	(95% CI)	N	OR	N	N	OR	(95% CI)		
<i>RPS6KBI</i> (rs180531)	<i>Akt1</i> (rs2494738)											
AA	913	708	1.00	GG	150	GA/AA	147	1.24	0.97,1.59			
AG	643	499	1.01		104		72	0.88	0.64,1.21			
GG	87	91	1.38		21		14	0.85	0.43,1.70			
P Interaction			0.03									
<i>RPS6KA1</i> (rs12025634)	<i>RPS6KA2</i> (rs3799620)											
CC/CT	1638	1293	1.00	AA/AG	287	GG	222	0.94	0.78,1.14			
TT	30	33	1.44		1		8	10.27	1.28,82.29			
P Interaction			0.02									
<i>RPS6KA2</i> (rs2072638)	<i>FRAP1</i> rs1057079											
TT	503	361	1.00	AA	462	AG/GG	338	0.98	0.81,1.20			
TC/CC	569	411	1.01		421		443	1.46	1.21,1.77			
p interaction			0.005									
<i>RPS6KA2</i> (rs395475) ²	<i>NFKB1</i> (rs4648110)											
TT	391	312	1.00	TT	225	TA/AA	202	1.12	0.88,1.43			
TC	628	484	0.96		326		261	1.01	0.81,1.26			
CC	227	204	1.12		162		96	0.73	0.54,0.98			
P Interaction			0.01									
Rectal Cancer	<i>PIK3CA</i> (rs7640662)											
<i>RPS6KBI</i> (rs180519)	CC											
GG	173	175	1.00		84	CG/GG	40	0.48	0.31,0.74			
GA	382	308	0.8		103		72	0.71	0.49,1.02			
AA	180	129	0.69		34		29	0.85	0.50,1.46			

¹ Adjusted for age, center, race/ethnicity, and sex.
² Same association seen with *NFKB1* (rs13117745)
³ Same association seen with *NFKB1* (rs13117745)

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Table 6

Combined genotypes and specific mutations in colon and rectal tumors

Colon Cancer											
CIMP High		KRAS2 Mutation				TP53 Mutation					
Frequency	OR ⁶	(95% CI)	Frequency	OR ⁶	(95% CI)	Frequency	OR ⁶	(95% CI)			
<i>RPS6KA2</i> ¹											
<i>RPS6KA1</i> and <i>RPS6KA2</i> ²											
0.32	C-T	0.87	(0.70, 1.06)	0.30	C-A-A-G-A	1.24	(1.02, 1.50)	0.50	A-A-G	0.95	(0.83, 1.10)
0.25	T-T	1.03	(0.82, 1.28)	0.13	C-G-A-G-A	0.80	(0.61, 1.05)	0.18	G-A-G	0.81	(0.67, 0.98)
0.24	C-C	0.91	(0.73, 1.14)	0.12	C-A-A-A-A	0.93	(0.71, 1.22)	0.15	A-A-A	1.31	(1.09, 1.59)
0.18	T-C	1.31	(1.04, 1.64)	0.07	T-A-A-G-A	1.12	(0.79, 1.58)	0.07	A-C-G	1.27	(0.97, 1.65)
				0.07	C-A-A-A-G	1.46	(1.07, 2.00)	0.05	G-A-A	0.77	(0.54, 1.12)
				0.05	C-A-C-G-G	0.96	(0.63, 1.48)	0.03	G-C-G	0.96	(0.64, 1.46)
				0.05	C-G-A-A-A	0.54	(0.31, 0.91)	0.02	A-C-A	0.99	(0.57, 1.73)
				0.04	T-A-A-A-A	0.98	(0.59, 1.62)				
				0.03	C-G-A-G-G	0.99	(0.63, 1.55)				
				0.03	C-G-C-G-G	1.06	(0.63, 1.78)				
				0.03	C-A-A-A-G	0.74	(0.40, 1.40)				
				0.02	T-A-A-G-G	1.16	(0.67, 2.01)				
				0.02	C-A-C-A-G	0.69	(0.32, 1.46)				
				0.01	C-G-A-A-G	0.37	(0.11, 1.19)				
Rectal Cancer											
<i>4RPS6KA2</i>											
<i>5RPS6KA2</i>											
0.36	T-A	0.69	(0.44, 1.08)	0.24	A-A-C	0.95	(0.71, 1.26)				
0.35	C-A	0.88	(0.58, 1.32)	0.21	A-C-C	0.92	(0.69, 1.23)				
0.20	T-G	0.83	(0.49, 1.42)	0.18	A-C-G	0.82	(0.61, 1.10)				
0.10	C-G	2.66	(1.65, 4.29)	0.14	A-A-G	1.27	(0.92, 1.75)				
				0.07	C-A-C	1.05	(0.65, 1.71)				
				0.07	C-C-C	0.71	(0.41, 1.23)				
				0.05	C-C-G	1.00	(0.57, 1.75)				
				0.03	C-A-G	2.55	(1.52, 4.26)				

¹ *RPS6KA2* rs10946164(C>T), rs2345067(T>C); minor allele in bold

- ² *RPS6KA1* rs12025634(C>T), rs12723936(A>G) and *RPS6KA2* rs16898963(G>A), rs409161(G>A), rs6926393(A>G); minor allele in bold
- ³ *RPS6KA1* rs12723936(A>G) and *RPS6KA2* rs16898963(A>C), rs17518299(G>A); minor allele in bold
- ⁴ *RPS6KA2* rs395475(T>C), rs6926393(A>G); minor allele in bold
- ⁵ *RPS6KA2* rs3816540(A>C), rs7760282(C>A), rs9347128(C>G); minor allele in bold
- ⁶ Adjusted for age, center, race/ethnicity, and sex; risk relative to all other haplotypes

Appendix

Description of *RPS6KA1*, *RPS6KA2*, *RPS6KB1*, *RPS6KB2*, and *PKD1*

Symbol	Location	SNP	MAF	Major/Minor Allele	FDR HWE Probability	Colon Homozygote Rare OR	Rectal Homozygote Rare OR
RPS6KA1	1p	rs12723936	0.27	A/G	1.00	0.81 (0.61, 1.08)	0.75 (0.49, 1.15)
		rs6666757	0.21	A/T	0.96	1.04 (0.74, 1.45)	0.81 (0.45, 1.45)
		rs737465	0.26	C/T	0.96	1.11 (0.85, 1.45)	1.17 (0.78, 1.77)
		rs2278978	0.26	G/A	0.98	1.17 (0.89, 1.53)	1.15 (0.76, 1.74)
		rs12025634	0.15	C/T	0.57	1.69 (1.05, 2.72)	1.03 (0.49, 2.17)
		rs17162190	0.21	G/A	1.00	1.01 (0.70, 1.45)	0.61 (0.37, 1.03)
		rs3816540	0.22	A/C	0.67	0.97 (0.70, 1.33)	0.83 (0.51, 1.37)
		rs11577405	0.30	G/T	0.67	0.94 (0.74, 1.20)	0.91 (0.62, 1.34)
		rs282175	0.28	T/C	1.00	0.85 (0.65, 1.12)	0.69 (0.45, 1.05)
		rs190737	0.47	T/G	0.81	0.97 (0.80, 1.17)	0.96 (0.71, 1.29)
		rs11113	0.45	T/C	<.01	1.08 (0.90, 1.31)	1.04 (0.77, 1.40)
		rs4709117	<.01	A/C	1.00	N/A	N/A
		rs11961547	0.17	A/T	0.91	1.20 (0.81, 1.79)	1.99 (1.00, 3.97)
		rs1927430	0.33	A/G	0.96	0.93 (0.73, 1.17)	0.86 (0.61, 1.21)
RPS6KA2	6q27	rs4710069	0.32	A/G	0.67	0.93 (0.73, 1.18)	0.69 (0.48, 0.98)
		rs9459678	0.33	T/C	0.96	1.25 (0.99, 1.59)	0.81 (0.57, 1.15)
		rs6456108	0.12	T/G	0.96	0.93 (0.53, 1.64)	0.41 (0.15, 1.13)
		rs3799620	0.36	A/G	0.99	0.92 (0.74, 1.15)	0.96 (0.68, 1.35)
		rs1202621	0.32	A/C	1.00	1.13 (0.89, 1.43)	1.24 (0.88, 1.74)
		rs3817782	0.41	G/A	1.00	1.09 (0.89, 1.35)	0.73 (0.54, 1.00)
		rs2187911	0.42	G/A	1.00	1.06 (0.87, 1.30)	0.92 (0.67, 1.25)
		rs909768	0.20	T/C	0.99	1.21 (0.86, 1.70)	1.27 (0.73, 2.21)
		rs12199759	0.19	A/G	0.96	1.35 (0.93, 1.96)	0.61 (0.32, 1.14)
		rs7760282	0.47	C/A	0.97	1.13 (0.93, 1.38)	1.30 (0.97, 1.73)
		rs2235296	0.32	C/T	0.97	1.07 (0.85, 1.36)	1.27 (0.89, 1.82)
		rs3799612	0.26	G/C	1.00	1.17 (0.89, 1.54)	1.01 (0.67, 1.53)
		rs6935542	0.13	A/G	1.00	1.25 (0.74, 2.09)	0.59 (0.20, 1.70)
		rs707768	0.45	G/A	1.00	1.10 (0.90, 1.34)	1.17 (0.87, 1.58)

Symbol	Location	SNP	MAF	Major/Minor Allele	FDR HWE Probability	Colon Homozygote Rare OR	Rectal Homozygote Rare OR
		rs763187	0.17	C/T	0.95	1.06 (0.71, 1.60)	0.95 (0.54, 1.67)
		rs6918886	0.42	G/A	0.98	1.08 (0.88, 1.32)	0.90 (0.66, 1.22)
		rs3799647	0.11	T/C	0.74	0.90 (0.50, 1.61)	0.83 (0.34, 2.03)
		rs13198549	0.37	A/G	1.00	0.99 (0.80, 1.24)	1.08 (0.78, 1.50)
		rs3799631	0.35	A/G	0.99	1.03 (0.82, 1.30)	1.21 (0.88, 1.68)
		rs9295348	0.29	A/C	0.97	1.15 (0.89, 1.48)	1.07 (0.74, 1.55)
		rs16898963	0.12	A/C	0.39	2.57 (1.37, 4.84)	0.95 (0.38, 2.38)
		rs1894660	0.18	G/A	0.96	0.88 (0.60, 1.30)	1.38 (0.77, 2.48)
		rs3823200	0.17	A/T	1.00	0.91 (0.60, 1.38)	0.70 (0.33, 1.46)
		rs6456103	0.06	G/A	0.91	0.93 (0.75, 1.15)*	0.98 (0.72, 1.34)*
		rs4709127	0.30	C/T	1.00	0.94 (0.73, 1.22)	0.82 (0.57, 1.19)
		rs10946164	0.44	C/T	1.00	1.09 (0.89, 1.33)	0.80 (0.59, 1.08)
		rs9348131	0.14	C/T	1.00	1.46 (0.87, 2.47)	1.03 (0.53, 2.00)
		rs2049957	0.49	T/C	0.99	1.01 (0.83, 1.23)	1.15 (0.85, 1.55)
		rs2049956	0.15	G/C	1.00	0.91 (0.58, 1.42)	1.41 (0.72, 2.78)
		rs6921577	0.44	A/G	0.78	1.09 (0.89, 1.33)	1.30 (0.96, 1.76)
		rs12200581	0.21	A/T	1.00	0.96 (0.68, 1.36)	0.90 (0.53, 1.53)
		rs6926393	0.29	A/G	1.00	1.26 (0.97, 1.63)	1.01 (0.70, 1.47)
		rs9366015	0.34	G/A	1.00	1.08 (0.86, 1.36)	0.81 (0.57, 1.15)
		rs10946174	0.29	G/A	0.96	0.92 (0.70, 1.20)	0.78 (0.50, 1.19)
		rs2071941	0.13	C/T	0.74	1.39 (0.83, 2.36)	1.09 (0.42, 2.86)
		rs411236	0.28	C/T	1.00	1.15 (0.88, 1.51)	0.97 (0.68, 1.40)
		rs395475	0.44	T/C	1.00	0.93 (0.76, 1.13)	0.95 (0.71, 1.28)
		rs384502	0.40	A/T	1.00	1.04 (0.85, 1.27)	1.17 (0.85, 1.60)
		rs3778405	0.10	A/G	0.96	1.08 (0.91, 1.29)*	1.13 (0.86, 1.48)*
		rs3778401	0.48	A/G	1.00	0.93 (0.77, 1.13)	1.01 (0.76, 1.35)
		rs763193	0.49	T/C	0.96	1.01 (0.83, 1.23)	0.71 (0.53, 0.95)
		rs9295355	0.29	T/A	0.91	0.95 (0.74, 1.21)	0.89 (0.59, 1.35)
		rs3799577	0.09	G/A	1.00	1.04 (0.86, 1.25)*	0.95 (0.73, 1.24)*
		rs1040446	0.11	T/G	1.00	0.98 (0.55, 1.74)	1.13 (0.38, 3.39)

Symbol	Location	SNP	MAF	Major/Minor Allele	FDR HWE Probability	Colon Homozygote Rare OR	Rectal Homozygote Rare OR
		rs6906202	0.26	T/C	0.96	1.03 (0.78, 1.38)	1.10 (0.73, 1.66)
		rs7766723	0.46	G/A	1.00	1.20 (0.98, 1.46)	1.11 (0.83, 1.49)
		rs7770200	0.13	A/G	1.00	1.12 (0.68, 1.83)	1.51 (0.70, 3.26)
		rs388033	0.20	T/C	0.85	1.19 (0.84, 1.68)	0.92 (0.52, 1.61)
		rs17518299	0.22	G/A	1.00	1.00 (0.72, 1.40)	1.28 (0.79, 2.07)
		rs12214750	0.17	A/C	1.00	0.87 (0.55, 1.37)	0.59 (0.31, 1.14)
		rs1309150	0.25	T/C	0.97	1.01 (0.75, 1.36)	0.74 (0.48, 1.14)
		rs6918384	0.20	C/T	0.39	0.76 (0.53, 1.09)	0.62 (0.35, 1.09)
		rs12208871	0.14	C/T	0.91	1.28 (0.75, 2.16)	1.56 (0.77, 3.15)
		rs9347128	0.39	C/G	1.00	1.13 (0.92, 1.39)	1.24 (0.91, 1.69)
		rs9295361	0.21	C/A	1.00	1.09 (0.79, 1.51)	1.42 (0.85, 2.36)
		rs932356	0.18	C/G	0.72	0.85 (0.59, 1.24)	1.03 (0.59, 1.79)
		rs9459715	0.11	T/G	0.67	0.51 (0.27, 0.96)	1.10 (0.37, 3.30)
		rs6911624	0.19	G/A	1.00	0.86 (0.58, 1.27)	0.67 (0.35, 1.30)
		rs1883361	0.42	C/T	1.00	1.05 (0.85, 1.28)	1.06 (0.79, 1.44)
		rs4710090	0.37	C/T	0.96	1.04 (0.83, 1.29)	0.92 (0.66, 1.27)
		rs388372	0.37	C/T	0.96	0.90 (0.72, 1.12)	0.99 (0.72, 1.36)
		rs409161	0.29	G/A	1.00	0.99 (0.76, 1.28)	0.85 (0.58, 1.24)
		rs661325	0.36	G/C	0.90	1.06 (0.84, 1.33)	0.98 (0.70, 1.38)
		rs2345067	0.43	T/C	1.00	1.08 (0.88, 1.32)	0.68 (0.50, 0.92)
		rs6456123	0.34	G/A	0.70	0.81 (0.63, 1.03)	1.03 (0.73, 1.46)
		rs2072638	0.30	T/C	0.25	1.18 (0.94, 1.48)	2.16 (1.52, 3.08)
		rs2072640	0.08	G/T	1.00	1.17 (0.96, 1.41)*	1.11 (0.82, 1.50)*
		rs7745781	0.12	A/G	1.00	1.12 (0.64, 1.95)	1.67 (0.78, 3.55)
		rs9348195	0.27	T/C	1.00	1.27 (0.97, 1.67)	0.98 (0.66, 1.44)
		rs8071475	0.25	T/C	0.89	1.00 (0.75, 1.35)	0.79 (0.50, 1.24)
		rs1292033	0.20	A/T	0.79	0.89 (0.62, 1.29)	1.12 (0.68, 1.85)
		rs180535	0.15	T/C	0.61	1.03 (0.67, 1.58)	0.64 (0.31, 1.34)
		rs180531	0.25	A/G	0.61	1.23 (0.92, 1.64)	0.70 (0.43, 1.13)
		rs180519	0.45	G/A	0.61	0.86 (0.70, 1.05)	0.92 (0.68, 1.25)
<i>RPS6KBI</i>	17q23.1						

Symbol	Location	SNP	MAF	Major/Minor Allele	FDR HWE Probability	Colon Homozygote Rare OR	Rectal Homozygote Rare OR
<i>RPS6KB2</i>	11q13.2	rs180515	0.36	A/G	0.89	0.89 (0.71, 1.11)	1.08 (0.78, 1.50)
		rs917570	0.44	C/G	0.89	0.91 (0.74, 1.11)	0.97 (0.72, 1.29)
		rs1638588	0.43	C/A	0.06	0.98 (0.79, 1.21)	0.89 (0.66, 1.19)
<i>PDK1</i>	2q31.1	rs11904366	0.15	G/T	1.00	1.20 (0.75, 1.90)	0.51 (0.22, 1.16)
		rs4972842	0.19	T/A	1.00	0.97 (0.66, 1.41)	1.31 (0.78, 2.19)
		rs11686903	0.35	C/T	1.00	1.03 (0.82, 1.30)	0.96 (0.68, 1.36)

* Indicates dominant model used due to MAF = 0.1.
Sample size ranges from 2723 to 2920
FDR = False Discovery Rate HWE= Hardy Weinberg Equilibrium test