Carcinogenesis vol.36 no.1 pp.49–59, 2015
doi:10.1093/carcin/bgu213
Advance Access publication October 20, 2014

A pathway approach to evaluating the association between the CHIEF pathway and risk of colorectal cancer

Martha L.Slattery*, Roger K.Wolff and Abbie Lundgreen

Department of Internal Medicine, University of Utah Health Sciences Center, Salt Lake City, UT 84108, USA

*To whom correspondence should be addressed. Department of Internal Medicine, University of Utah Health Sciences Center, 383 Colorow Building, Salt Lake City, UT 84108, USA. Tel: +1 801 585 6955; Fax: +1 801 581 3623;
Email: marty.slattery@hsc.utah.edu

Inflammation, hormones and energy-related factors have been associated with colorectal cancer (CRC) and it has been proposed that convergence and interactions of these factors importantly influence CRC risk. We have previously hypothesized that genetic variation in the CHIEF (convergence of hormones, inflammation and energy-related factors) pathway would influence risk of CRC. In this paper, we utilize an Adaptive Rank Truncation Product (ARTP) statistical method to determine the overall pathway significance and then use that method to identify the key elements within the pathway associated with disease risk. Data from two population-based case–control studies of colon (n = 1555 cases and 1956 controls) and rectal (n = 754 cases and 959 controls) cancer were used. We use ARTP to estimate pathway and gene significance and polygenic scores based on ARTP findings to further estimate the risk associated with the pathway. Associations were further assessed based on tumor molecular phenotype. The CHIEF pathway was statistically significant for colon cancer (P_{ARTP} = 0.03) with the most significant interferons (P_{ARTP} = 0.0253), JAK/STAT/SOCS (P_{ARTP} = 0.0111), telomere (P_{ARTP} = 0.0399) and transforming growth factor β (P_{ARTP} = 0.0043) being the most significant subpathways for colon cancer. For rectal cancer, interleukins (P_{ARTP} = 0.0235) and selenoproteins (P_{ARTP} = 0.0047) were statistically significant although the pathway overall was of borderline significance (P_{ARTP} = 0.06). Interleukins (P_{ARTP} = 0.0456) and mitogen-activated protein kinase (P_{ARTP} = 0.0392) subpathways were uniquely significant for CpG island methylator phenotype-positive colon tumors. Increasing number of at-risk alleles was significantly associated with both colon [odds ratio (OR) = 6.21, 95% confidence interval (CI): 4.72, 8.16] and rectal (OR = 7.82, 95% CI: 5.26, 11.62) cancer. We conclude that elements of the CHIEF pathway are important for CRC risk.

Introduction

Inflammatory processes appear to be a key element in the underlying carcinogenic process for colorectal cancer (CRC). We proposed an integrated pathway where the convergence of hormones, inflammation and energy-related factors (CHIEF) are central to the etiology of CRC (1). The CHIEF pathway takes an integrated approach to CRC through its incorporation of elements of angiogenesis, hormones and energy-related factors within the underlying inflammatory state of the colon and rectum (see Figure 1).

Abbreviations: ARTP, Adaptive Rank Truncation Product; CHIEF, convergence of hormones, inflammation and energy-related factors; CI, confidence interval; CIMP, CpG island methylator phenotype; CRC, colorectal cancer; KPMCP, Kaiser Permanente Medical Care Program of Northern California; LD, linkage disequilibrium; MAPK, mitogen-activated kinase; MSI, microsatellite instability; MTOR, mammalian target of rapamycin; NF-κB, nuclear factor kappa B; OR, odds ratio; SNP, single nucleotide polymorphism; STAT, signal transduction and activation of transcription; TGFβ, transforming growth factor β; TLR, Toll-like receptor; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

Genes within the pathway function within multiple subpathways (a list of all of the genes included along with their alias and chromosome location is given in Supplementary Table 1, available at *Carcinogenesis* Online). For instance, the pathway core contains a serine/threonine protein kinase 11 (STK11 or LKB1) and is involved in the regulation of mammalian target of rapamycin (MTOR). STK11 responds to changes in cellular energy balance (ATP levels) (2,3) and governs whole body insulin sensitivity (4,5). A different portion of the pathway that responds to insulin, estrogen and androgen and certain proto-oncogene growth factors contain the tumor suppressor PTEN (Phosphatase TENsin homolog deleted on chromosome 10). PTEN acts as a metabolic regulator by modulating signaling via the phosphatidylinositol 3-kinase (PI3K; oncogene formal name *PIK3CA*) and the v-akt murine thymoma viral oncogene homolog 1 (*Akt1* also known as protein kinase B or *PKB*) pathway. Nuclear factor kappa B (NF-κB) is an important nuclear transcription factor that regulates cytokines and is critical for the regulation of tumorigenesis, cell proliferation, apoptosis, response to oxidative stress and inflammation. Vascular endothelial growth factor (VEGF) regulates RPS6K and IRS-1 and plays an important role in regulation of cell growth signaling; it is a major mediator of tumor angiogenesis (6,7). Steroid hormones including estrogen, androgen and progesterone have been shown to have both anti- and proinflammatory properties (8,9). The receptors of the steroid hormones have been shown to interact with NF-κB in an antagonist manner (9–11). Estrogen also has been shown to repress IL-6 expression as well as IκB, potentially explaining its anti-inflammatory mechanism (9,12).

Cytokines are a key element of the inflammatory process in the CHIEF pathway since inflammation is initiated by the synthesis and secretion of proinflammatory cytokines [e.g. tumor necrosis factor (TNF) and IL-6 in macrophages] in response to an inflammation-provoking insult. The binding of proinflammatory cytokines to their receptors triggers the activation of NF-κB, which in turn activates the expression of a wide variety of genes including cytokines and cyclooxygenase-2 (COX-2). The transforming growth factor β (TGFβ) signaling pathway is involved in all aspects of tumorigenesis, including cell growth regulation, avoidance of apoptosis, promotion of inflammation and angiogenesis, immune response and stimulation of tumor invasion and metastasis (13). Key cytokine-related genes are interleukins, interferons and TNF-related genes and genes within the TGFβ signaling pathway.

Included in the CHIEF pathway are signal transduction and activation of transcription (STAT) and mitogen-activated kinases (MAPKs) genes that are involved in both inflammation and metabolic signaling associated with hormones and energy-related factors. The STAT protein family members are phosphorylated in response to cytokines and growth factors and involved in convergent areas of multiple pathways. MAPKs serve as an integration point for multiple biological signals and are involved in a variety of cellular processes such as proliferation, differentiation and transcription regulation and have three primary components—JNK, p38 and ERK. JNK-1 or MAPK-8 is activated by TNF-α and is necessary for apoptosis. NF-κB is required to terminate JNK signaling. P38, also known as MAPK-14, plays a role in multiple mechanisms including angiogenesis, the PI3K pathway and cytokines. The inflammatory loci are further influenced by interaction with epithelial and vascular endothelial cells and are closely linked with angiogenesis, another component of the CHIEF pathway; angiogenesis and inflammation are hallmark features of tumorigenesis (14).

In this paper, we summarize the significance of this pathway as it relates to colon and rectal cancer risk using Adaptive Rank Truncation Product (ARTP). This statistical method utilizes a permutation method that allows us to combine single nucleotide polymorphism (SNP) P values within a gene, gene ARTP P values within a subpathway and

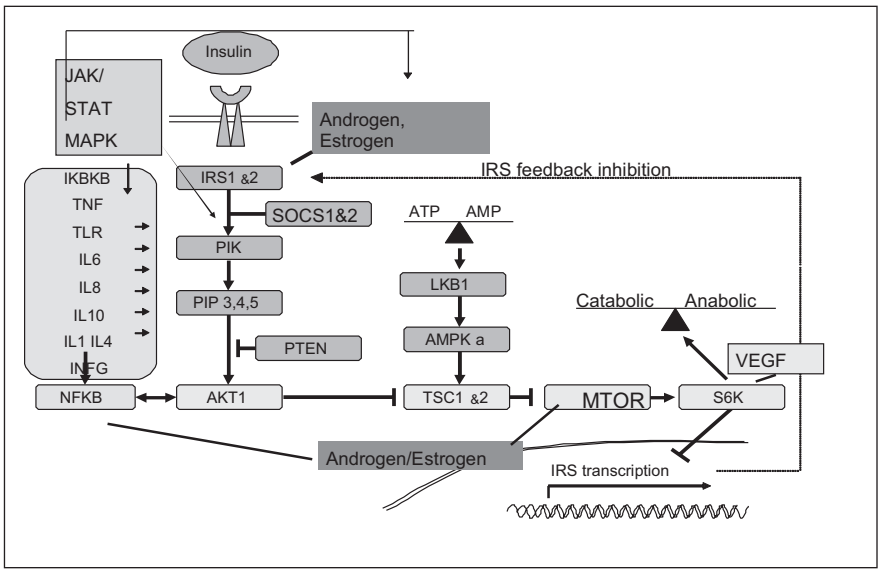

Fig. 1. Convergent signaling pathways where inflammation and metabolic signaling intersect along the CHIEF pathway.

subpathway ARTP *P* values within a pathway in order to capture gene, subpathway and pathway level effects with colon and rectal cancer. To further estimate the magnitude of the association of this pathway on colon and rectal cancer risk, we utilize a polygenic risk score that is based on the ARTP findings. We evaluate associations overall as well as by tumor molecular phenotype.

Methods

Two study populations are included in these analyses. The first study, a population-based case–control study of colon cancer, included cases (n = 1555 with complete genotype data) and controls (n = 1956 with complete genotype data) identified between 1 October 1991 and 30 September 1994, 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) (15). 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 (16). 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 previous 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 since diet history questionnaire was not adapted at that time for other ethnic groups). 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. In Minnesota, controls were selected from driver's license and state-identification lists. Eligibility for controls was the same as those outlined for cases. Study details have been previously reported (15,16). 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.

TagSNPs and genetic assessment

TagSNPs were selected using the following parameters: r^2 = 0.8 defined linkage disequilibrium (LD) blocks using a Caucasian LD map, minor allele frequency >0.1, range = −1500 bp from the initiation codon to +1500 bp from the termination codon and one SNP/LD bin. All markers were genotyped using a custom multiplexed bead array assay format 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 sample set. The duplicate concordance rate was 100.00%. Supplementary Table 1, available at *Carcinogenesis* Online, list all genes included in the subpathway, whereas Supplementary Table 2, available at *Carcinogenesis* Online, list number of SNPs assessed for each gene and the P_{ARTP} value for each gene on the platform. A total of 155 genes and 1246 SNPs were included in the analysis. SNPs per gene ranged from 1 to 71.

Statistical methods

The LD measure, minor allele frequency and test for Hardy–Weinberg equilibrium were calculated among White controls using the ALLELE procedure within SAS version 9.3 (SAS Institute, Cary, NC). The goal of the analysis was to evaluate the overall associations between genes and pathways as they relate to colon and rectal cancer. To do this, we used ARTP which utilizes a highly efficient permutation algorithm to determine significance at the gene, subpathway and pathway level for colon and rectal separately (17). Case/control status was permuted 10 000 times within R version 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria) and *P* values based on 1-degree of freedom (df) Wald chi-square tests were generated from logistic regression models adjusted for age, study center, race/ethnicity and sex. Associations with SNPs within ARTP were assessed assuming an additive model unless a preliminary check of the odds ratios (ORs) using the co-dominant model indicated a dominant or recessive mode of inheritance. For SNPs with *P* values <0.05 on genes that were associated with colon or rectal cancer using ARTP, we also report ORs and 95% confidence intervals (CIs) assessed from multiple logistic regression models in SAS, adjusting for study matching variables: age, center, race/ethnicity and sex to show the magnitude of the association between these SNPs and colon or rectal cancer risk. Genes were assigned to only one subpathway prior to the hierarchical analyses, although many genes could function in multiple subpathways.

Tumors were defined by specific molecular alterations: any *TP53* mutation, any *KRAS* mutation, MSI+ and CpG island methylator phenotype (CIMP) which was defined as positive if at least two of five markers methylated. CIMP was based on the classic panel (18). Microsatellite instability (MSI) was based on *BAT26*, *TGFβRII* and a panel of 10 tetranucleotide repeats that has been shown to correlate highly with the Bethesda Panel (19); our study was done prior to the Bethesda Panel development. As the proportion of MSI+ tumors in the rectal cases was <3% (20), we did not examine these tumor markers. We evaluated pathway associations using ARTP as described above for tumor molecular phenotypes relative to controls.

To summarize the risk associated with the CHIEF pathway, we calculated polygenic summary scores. To conservatively estimate risk, we included, in the risk models, SNPs from genes where the gene ARTP *P* values were ≤0.10 and the SNP *P* values within those genes were ≤0.10. Since genes are associated with multiple subpathways, we did not restrict to genes where the subpathway was significant. If SNPs within the same gene had r^2 values of ≥0.80, only one SNP was included in the model. Risk was modeled using at-risk alleles, using all genotypes with the low-risk genotype or referent group as zero. For the co-dominant or additive model, a score of 0, 1 or 2 was assigned relative to the number of at-risk alleles, whereas scores of 0 or 2 were assigned for the dominant and recessive models in order to capture the risk associated with the various genotypes. Polygenic scores were then used to summarize risk across the genes and SNPs to better capture the risk associated with the pathway. Individuals missing >5% of SNP data were dropped from the analysis. The continuous score variable was redefined as a categorical variable based on

number of at-risk alleles and the distribution of the populations. Adjustments for body mass index, family history of CRC, use of aspirin/non-steroidal anti-inflammatory drugs, cigarette smoking status and dietary energy intake did not alter the observed risk, therefore adjustments were made for matching variables of age, sex, race and study center only. All of the genes assessed and their corresponding subpathway and gene P_{ARTP} are included in Supplementary Table 2, available at *Carcinogenesis* Online.

Results

The majority of cases and controls were over 60 years of age, were non-Hispanic white and were male (Table I). *TP53* mutations were slightly more prevalent among rectal cancer cases (49.64%) versus colon cancer cases (45.95%). MSI tumors were rare among rectal cancers, and CIMP+ tumors were present in 11.11% of rectal cancers and in 26.93% of colon cancers.

Overall, the CHIEF pathway was statistically significant for colon cancer (P_{ARTP} = 0.03) and of borderline significance for rectal cancer (P_{ARTP} = 0.06) (Table II). The most significant subpathways for colon cancer were interferons (P_{ARTP} = 0.0253), JAK/STAT/SOCS (P_{ARTP} = 0.0111), telomere (P_{ARTP} = 0.0399) and TGFβ signaling (P_{ARTP} = 0.0043). For rectal cancer, interleukins (P_{ARTP} = 0.0235) and selenoproteins (P_{ARTP} = 0.0047) were statistically significant.

Genes with a P_{ARTP} <0.10 and their related SNPs with a P <0.10 are shown in Table III for colon cancer and in Table IV for rectal cancer. Of the 1246 SNPs assessed, 116 were on genes with a P_{ARTP} <0.1 and were associated with colon cancer at the 0.10 level (5 excluded from score because of LD >0.80: *IL6R* rs8192284, *IL8* rs2227307, *JAK2* rs2072593, *MTOR* rs2295080 and rs718206) and 70 for rectal cancer (15 excluded from score because of high LD: *VEGFA* rs833069, *IL8RA* rs4674258, *MAPK8* rs10857561, *NFKB1* rs3774932, rs1801, rs4648068, *PIK3CA* rs12509517, rs3755867 and rs1609798, *SEPN1* rs4419933, *BMPR2* rs6796916). Several significant genes within subpathways were detected, even though the overall subpathway was not significant. For colon cancer, 8 of the 24 genes that were significantly associated with risk at the 0.05 level were in the TGFβ signaling pathway. Three genes in the Jak/Stat/Socs subpathway (*STAT3*, *STAT5A* and *STAT5B*) and three MAPK genes (*MAP2K1*, *MAP3K3* and

Table I. Description of study population

	Colon		Rectal	
	Controls	Cases	Controls	Cases
	n (%)	*n* (%)	*n* (%)	*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)		
Race/ethnicity				
Non-Hispanic white	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			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)
Tumor molecular phenotypes				
CIMP+		272 (26.93)		59 (11.11)
KRAS2 Mutation		348 (31.90)		173 (29.37)
TP53 Mutation		516 (45.95)		277 (49.64)
MSI Unstable		185 (15.74)		14 (2.39)

Table II. Associations between CHIEF pathway and colon and rectal cancer overall and by tumor molecular phenotype

Sub-Pathway	Colon cancer		Rectal cancer	
	Subpathway	Overall pathway	Subpathway	Overall pathway
	P value	*P* value	*P* value	*P* value
Angiogenesis	0.703	**0.030**	0.402	0.064
Hormone/insulin/growth	0.077		0.790	
Interferon	**0.025**		0.379	
Interleukin	0.123		**0.024**	
Jak/Stat/Socs	**0.011**		0.471	
Pathway core	0.183		0.165	
MAPK	0.164		0.368	
Selenoprotein	0.856		**0.0047**	
Telomere	**0.040**		0.122	
TGFβ	**0.004**		0.516	
TLR	0.223		0.428	
TNF	0.151		0.204	

Bold values indicate significant at <0.05 level.

Table III. Associations between subpathway genes and colon cancer for SNPs included in polygenic score ($P_{ARTP} < 0.10$)

Subpathway	Gene	Gene P_{ARTP}	SNP	Genotype	SNP P[a]	OR 95% CI[b]
Angiogenesis	*FLT1*	0.089	rs2296189	AG/GG versus AA	0.029	0.85 (0.74, 0.98)
			rs3794400	TT versus CC	0.012	1.36 (0.94, 1.96)
			rs2387632	TT versus CC/CT	0.007	1.32 (1.08, 1.62)
			rs1324057	TT versus CC	0.031	1.26 (0.99, 1.60)
			rs7324547	GA/AA versus GG	0.033	1.16 (1.01, 1.34)
			rs9513088	GG versus AA	0.050	1.22 (0.99, 1.51)
			rs2296283	CC versus TT	0.072	1.20 (0.99, 1.45)
			rs12858139	CA/AA versus CC	0.010	1.21 (1.05, 1.40)
			rs600640	TC/CC versus TT	0.041	1.15 (1.01, 1.32)
			rs678714	TA/AA versus TT	0.020	0.82 (0.69, 0.97)
Hormone/insulin/ growth	***SLC2A4***	0.010	rs5435	TT versus CC/CT	0.001	0.73 (0.60, 0.88)
	TCF7L2	0.066	TCF7L2	CT/TT versus CC	0.068	1.13 (0.99, 1.30)
	VDR	0.018	VDR_Fok1	ff versus FF	0.005	0.75 (0.60, 0.93)
			VDR_Poly	SS versus LL	0.036	0.79 (0.65, 0.97)
Interferons	***IFNG***	0.003	rs2069718	TT versus CC	0.070	0.84 (0.69, 1.02)
			rs1861493	AG/GG versus AA	0.001	0.79 (0.69, 0.91)
	IRF3	0.012	rs2304204	GG versus AA/AG	0.004	1.43 (1.12, 1.82)
Interleukins	*IL15*	0.061	rs1519551	GG versus AA	0.033	0.81 (0.67, 0.99)
			rs13117878	TT versus CC	0.011	1.28 (1.06, 1.55)
	IL2RA	0.095	rs12244380	AG/GG versus AA	0.043	1.16 (1.00, 1.34)
			rs12722596	GG versus AA	0.075	1.34 (0.66, 2.69)
			rs12722561	AA versus GG/GA	0.004	0.49 (0.30, 0.79)
			rs706779	GG versus AA	0.062	1.19 (0.99, 1.43)
			rs3118470	TC/CC versus TT	0.043	0.87 (0.76, 1.00)
	IL3	0.056	rs181781	AA versus GG/GA	0.025	2.42 (1.12, 5.22)
	IL6	0.095	rs1800795	CC versus GG	0.073	0.84 (0.69, 1.03)
			rs2069860	AT/TT versus AA	0.033	0.55 (0.32, 0.95)
	IL6R	0.033	rs7549250	TC/CC versus TT	0.026	0.85 (0.74, 0.98)
			rs4845623	GG versus AA/AG	0.005	1.29 (1.08, 1.53)
	IL8	0.027	rs4073	AA versus TT	0.014	1.26 (1.04, 1.53)
Jak/Stat/Socs	*JAK2*	0.072	rs1887429	GT/TT versus GG	0.029	0.86 (0.75, 0.99)
			rs3780379	AA versus GG/GA	0.007	1.68 (1.16, 2.44)
	SOCS2	0.057	rs768775	TC/CC versus TT	0.020	1.18 (1.03, 1.36)
	STAT3	0.024	rs8069645	AG/GG versus AA	0.031	1.16 (1.01, 1.32)
			rs6503695	CC versus TT	0.003	1.32 (1.07, 1.64)
			rs12949918	CC versus TT	0.023	1.23 (1.01, 1.49)
			rs1026916	AA versus GG	0.064	1.20 (0.98, 1.48)
	STAT5A	0.008	rs7217728	CC versus TT	0.003	1.31 (1.04, 1.66)
	STAT5B	0.007	rs6503691	TT versus CC	0.007	1.59 (1.00, 2.53)
			rs7218653	GG versus AA	0.009	1.30 (1.03, 1.64)
	STAT6	0.095	rs324015	GA/AA versus GG	0.020	0.85 (0.74, 0.97)
			rs324011	TT versus CC	0.087	1.20 (0.98, 1.46)
MAPK	*DUSP2*	0.093	rs1724120	AA versus GG/GA	0.095	1.15 (0.98, 1.35)
	MAP2K1	0.043	rs1432442	AA versus GG	0.075	0.69 (0.35, 1.38)
			rs7181936	TT versus GG/GT	0.027	1.27 (1.03, 1.58)
			rs8039880	GG versus AA	0.007	0.57 (0.38, 0.83)
	MAP3K3	0.035	rs11658329	GC/CC versus GG	0.035	1.16 (1.01, 1.32)
			rs3785574	GG versus AA/AG	0.031	0.79 (0.63, 0.98)
	MAP3K9	0.018	rs11625206	TT versus CC/CT	0.002	1.41 (1.14, 1.76)
			rs11844774	TC/CC versus TT	0.008	0.83 (0.72, 0.95)
			rs11628333	CC versus TT/TC	0.028	1.25 (1.02, 1.52)
			rs17176971	AA versus GG/GA	0.041	1.49 (1.02, 2.20)
			rs11624934	GG versus AA/AG	0.002	1.42 (1.13, 1.79)
Pathway core	***MTOR***	0.012	rs1057079	AG/GG versus AA	0.006	1.21 (1.06, 1.38)
			rs2024627	CT/TT versus CC	0.031	1.16 (1.01, 1.33)
	PRKAG2	0.072	rs1029947	GG versus AA/AG	0.046	1.70 (1.01, 2.87)
			rs6464156	AG/GG versus AA	0.020	0.83 (0.71, 0.97)
			rs953221	CT/TT versus CC	0.033	0.86 (0.75, 0.99)
			rs9648723	CC versus AA/AC	0.013	1.51 (1.09, 2.09)
			rs6947064	GG versus AA	0.061	0.81 (0.66, 1.00)
			rs2374270	AA versus CC	0.069	0.83 (0.67, 1.03)
			rs1860743	AA versus GG	0.018	0.65 (0.36, 1.16)
			rs2536068	AA versus GG	0.072	1.18 (0.84, 1.66)
			rs1104897	AA versus GG	0.009	1.46 (0.94, 2.26)
			rs6965771	TT versus CC	0.090	0.89 (0.69, 1.16)
			rs9632641	CC versus AA	0.023	1.28 (0.92, 1.78)
			rs6464170	GG versus CC/CG	0.038	0.73 (0.54, 0.98)
			rs9648724	AA versus GG	0.022	0.74 (0.56, 1.00)
			rs12703162	CT/TT versus CC	0.049	0.87 (0.75, 1.00)
	RPS6KB2	0.010	rs917570	CG/GG versus CC	0.004	0.81 (0.70, 0.94)

Table III. *Continued*

Subpathway	Gene	Gene P_{ARTP}	SNP	Genotype	SNP P^a	OR 95% CI[b]
Telomere	***TERT***	0.040	rs2853668	AA versus CC	0.036	0.70 (0.54, 0.92)
			rs2736118	GG versus AA	0.048	1.31 (1.02, 1.69)
			rs2736100	GG versus TT/TG	0.016	0.83 (0.71, 0.97)
TGFβ	***BMP2***	0.002	rs1979855	TC/CC versus TT	0.001	1.29 (1.11, 1.48)
			rs3178250	TC/CC versus TT	0.009	1.20 (1.05, 1.38)
			rs235770	TT versus CC	0.022	0.78 (0.63, 0.97)
	BMPR1A	0.038	rs6586034	TG/GG versus TT	0.010	0.82 (0.71, 0.95)
			rs7088641	TC/CC versus TT	0.006	0.83 (0.73, 0.95)
	BMPR1B	0.017	rs9307147	GG versus AA	0.003	0.75 (0.62, 0.91)
			rs4490463	GG versus AA	0.038	0.82 (0.67, 1.00)
			rs17616243	TT versus CC	0.006	1.45 (0.94, 2.26)
			rs2120834	CC versus GG/GC	0.019	0.79 (0.65, 0.96)
			rs7662504	CC versus AA	0.017	0.78 (0.64, 0.96)
			rs7694043	CT/TT versus CC	0.095	1.12 (0.98, 1.29)
			rs13134042	AA versus GG	0.081	0.69 (0.49, 0.97)
			rs1863652	TT versus CC/CT	0.030	0.79 (0.64, 0.98)
	EIF4E	0.007	rs12498533	CC versus AA	0.013	1.25 (1.03, 1.52)
			rs11727086	GG versus AA	0.004	1.33 (0.99, 1.77)
	RUNX1	0.090	rs2834645	TC/CC versus TT	0.006	0.82 (0.72, 0.95)
			rs2834650	CT/TT versus CC	0.066	0.85 (0.71, 1.01)
			rs2268281	GG versus AA	0.076	1.40 (0.96, 2.03)
			rs2248720	AC/CC versus AA	0.053	0.86 (0.74, 1.00)
			rs2252585	CC versus TT	0.057	1.20 (0.94, 1.53)
			rs8134179	CC versus TT	0.060	0.71 (0.48, 1.06)
			rs2834670	GG versus AA	0.097	1.14 (0.85, 1.53)
			rs2242878	CT/TT versus CC	0.011	1.20 (1.04, 1.38)
			rs7279123	CT/TT versus CC	0.015	1.18 (1.03, 1.35)
	SMAD2	0.044	rs1787199	TT versus AA	0.028	1.24 (1.03, 1.51)
			rs4940086	CC versus TT	0.019	1.33 (1.06, 1.66)
	SMAD3	0.015	rs12904944	AA versus GG	0.069	0.81 (0.64, 1.01)
			rs1498506	CC versus AA	0.0003	0.69 (0.57, 0.84)
			rs12901071	GG versus AA	0.004	0.67 (0.53, 0.84)
			rs2414937	CC versus GG/GC	0.034	0.68 (0.47, 0.97)
	SMAD7	0.045	rs4939827	TC/CC versus TT	0.028	0.84 (0.73, 0.98)
			rs12953717	TT versus CC	0.003	1.36 (1.12, 1.65)
	TGFB1	0.000	rs4803455	AA versus CC	0.0003	1.43 (1.18, 1.74)
			rs1800469	AA versus GG	0.001	0.66 (0.52, 0.85)
	TGFBR1	0.087	rs6478974	TA/AA versus TT	0.037	0.85 (0.74, 0.99)
			rs1571590	GG versus AA/AG	0.049	1.41 (1.00, 1.98)
TNF	***TNF***	0.036	rs1799964	CC versus TT	0.055	1.29 (0.94, 1.76)
			rs1800630	CA/AA versus CC	0.020	1.19 (1.03, 1.38)

Genes with P_{ARTP} <0.05 are indicated in bold.
[a]*P* values based on 1-df Wald chi-square tests.
[b]Models adjusted for age, study center, race/ethnicity and sex.

MAP3K9) were significantly associated with colon cancer. *SLC2A4* (alias *GLUT4*) and *VDR* were the only hormone/insulin-related genes associated with colon cancer. *MTOR* and *RPS6KB2* were significantly associated with colon cancer as was *TERT*. Other genes that showed a significant association with colon cancer were *IFGN*, *IRF3*, *IL6R*, *IL8* and *TNF*. An additional 34 SNPs had SNP P_{ARTP} <0.05 although the gene P_{ARTP} was between 0.05 and 0.10.

Fewer genes and SNPs were significantly associated with rectal cancer (Table IV). Genes in seven subpathways were statistically significant at the P_{ARTP} <0.05, whereas another 13 genes had P_{ARTP} values between 0.05 and 0.10. However, in this last group of genes, there were 24 SNPs with *P* values of <0.05. The interleukin and selenoprotein subpathways were more significantly associated with rectal cancer than colon cancer, although TGFβ signaling pathway had less of an impact on rectal cancer.

There were few subpathways associated with specific tumor molecular phenotype (Table V). Interleukins (P_{ARTP} = 0.0456) and MAPK (P_{ARTP} = 0.0392) subpathways were significant for CIMP+ colon tumors, while not significant for overall colon cancers; none of the subpathways were associated with MSI tumors. However, even though the overall subpathways were not statistically significant at the 0.05 level, there were several genes within these subpathways that had P_{ARTP} values <0.05.

To summarize the risk associated with the CHIEF pathway, we report polygenic summary scores (see Figure 2). With increasing number of at-risk alleles, there was a significant increase in risk of both colon and rectal cancer. The magnitude of risk was slightly greater for rectal cancer (OR: 7.82, 95% CI: 5.26, 11.62) than for colon cancer (OR: 6.21, 95% CI: 4.72, 8.16), despite the fact that more SNPs were included in the risk score for colon cancer than for rectal cancer.

Discussion

The physiological structure of the gut and supportive epidemiological and molecular data led us to propose that basal immune activation, a repetitive, mild subclinical inflammation, is the underlying modulator of CRC risk and influences the CRC risk associated with insulin, estrogen and energy-related factors. Overall, the CHIEF pathway was statistically significant for colon cancer and marginally significant for rectal cancer. While subpathways within the overall pathway indicate areas of importance, specific genes within pathway also are important. This is not surprising since these genes work in multiple pathways and could contribute to risk through their involvement in other subpathways than the one in which they were evaluated. Additionally, pathways of significance appear to differ for colon and rectal cancer. The TGFβ signaling pathway was most important for colon cancer, whereas the interleukin and selenoprotein subpathways

Table IV. Associations between subpathway genes and SNPs with rectal cancer that were used in polygenic score ($P_{ARTP} < 0.10$)

Subpathway	Gene	Gene P_{ARTP}	SNP	Genotype	SNP P[a]	OR 95% CI[b]
Angiogenesis	***MPO***	0.041	rs2243828	AG/GG versus AA	0.039	0.81 (0.67, 0.99)
	VEGFA	0.057	rs2010963	GC/CC versus GG	0.007	1.30 (1.07, 1.58)
Hormone/insulin/ growth	*IGF1R*	0.079	IGF1R	CC versus AA	0.078	1.51 (0.93, 2.43)
Interferons	*IFNGR1*	0.052	rs3799488	CC versus TT/TC	0.040	2.30 (1.04, 5.08)
			rs9376267	CT/TT versus CC	0.065	1.20 (0.99, 1.46)
	IRF2	0.072	rs3775554	CC versus GG	0.057	1.06 (0.46, 2.44)
			rs3775556	GG versus AA	0.007	1.48 (1.00, 2.20)
			rs10009261	TT versus CC	0.038	0.77 (0.57, 1.04)
			rs7677486	TT versus CC	0.003	1.54 (1.16, 2.03)
			rs6827018	GG versus AA	0.032	1.70 (0.75, 3.83)
			rs3733473	AA versus GG	0.078	0.97 (0.61, 1.53)
			rs3775574	AG/GG versus AA	0.003	0.74 (0.60, 0.90)
Interleukins	*IL15*	0.064	rs12508866	TC/CC versus TT	0.019	0.79 (0.65, 0.96)
			rs17461269	TA/AA versus TT	0.025	1.25 (1.03, 1.52)
			rs13117878	TT versus CC	0.066	1.29 (0.98, 1.69)
	IL8RA	0.006	rs1008562	GG versus CC	0.001	1.57 (1.20, 2.05)
	IL8RB	0.002	rs1126579	TT versus CC	0.001	1.56 (1.19, 2.05)
Jak/Stat/Socs	*STAT3*	0.083	rs2293152	CC versus GG/GC	0.014	0.71 (0.54, 0.93)
	STAT6	0.100	rs3024979	TA/AA versus TT	0.016	0.73 (0.56, 0.94)
MAPK	***DUSP1***	0.030	rs322351	TT versus CC	0.013	1.43 (1.09, 1.88)
	DUSP7	0.051	rs9851576	AG/GG versus AA	0.054	0.81 (0.66, 1.00)
	MAPK8	0.055	rs10508901	AA versus CC/CA	0.023	1.45 (1.05, 1.99)
Pathway core	***NFKB1***	0.017	rs230510	TT versus AA	0.006	0.67 (0.50, 0.90)
			rs3821958	GG versus AA	0.043	1.35 (1.02, 1.78)
			rs11722146	AA versus GG	0.015	1.42 (1.02, 1.97)
	PIK3CA	0.077	rs2699905	GA/AA versus GG	0.054	0.82 (0.67, 1.00)
			rs7651265	GG versus AA	0.063	2.38 (1.04, 5.43)
			rs7640662	GG versus CC	0.012	0.30 (0.11, 0.79)
	PIK3CG	0.019	rs4460309	TT versus CC	0.005	0.66 (0.43, 1.00)
			rs11766675	GG versus AA	0.003	0.57 (0.34, 0.96)
	RPS6KA2	0.072	rs10946164	CT/TT versus CC	0.020	0.78 (0.64, 0.96)
			rs9347128	GG versus CC/CG	0.027	1.32 (1.03, 1.68)
			rs1040446	TG/GG versus TT	0.038	0.78 (0.61, 0.99)
			rs6911624	AA versus GG	0.002	0.55 (0.31, 0.98)
			rs4709127	TT versus CC	0.047	0.74 (0.52, 1.06)
			rs2345067	CC versus TT	0.035	0.75 (0.57, 0.98)
			rs1202621	CC versus AA	0.073	1.25 (0.93, 1.68)
			rs7745781	AG/GG versus AA	0.001	1.42 (1.15, 1.76)
	RPS6KB2	0.057	rs1638588	AA versus CC	0.028	0.73 (0.56, 0.96)
	TSC1	0.043	rs13295634	GG versus TT/TG	0.004	0.61 (0.43, 0.86)
Selenoproteins	***SEPN1***	0.002	rs718391	GG versus CC	0.010	0.70 (0.53, 0.93)
			rs2072749	GG versus AA	0.003	0.53 (0.35, 0.80)
			rs11247735	AA versus GG/GA	0.021	1.31 (1.04, 1.64)
			rs4659382	GG versus CC/CG	0.007	0.58 (0.39, 0.86)
	SEPP1	0.092	rs3877899	AA versus GG	0.039	0.68 (0.43, 1.07)
			rs11959466	GA/AA versus GG	0.019	1.46 (1.06, 2.00)
			rs28919882	AG/GG versus AA	0.023	0.72 (0.55, 0.96)
	TXNRD3	0.004	rs4679274	TT versus CC/CT	0.034	0.73 (0.54, 0.98)
			rs9637365	TT versus CC/CT	0.006	0.70 (0.55, 0.90)
			rs11718498	GA/AA versus GG	0.001	1.42 (1.15, 1.74)
TGFβ	***BMPR2***	0.026	rs4675278	AA versus GG	0.087	1.29 (0.92, 1.81)
			rs17199235	AG/GG versus AA	0.018	1.35 (1.05, 1.72)
			rs2228545	GA/AA versus GG	0.002	1.93 (1.28, 2.91)
TNF	***NFAT5***	0.030	rs12447326	TT versus CC/CT	0.003	0.60 (0.43, 0.84)
			rs16959025	GG versus TT/TG	0.025	0.57 (0.35, 0.93)

Significant genes with P_{ARTP} <0.05 are indicated in bold.
[a]*P* values based on 1-df Wald chi-square tests.
[b]Models adjusted for age, study center, race/ethnicity and sex.

were important for rectal cancer. Although a greater number of genes and SNPs were associated with colon cancer, the risk associated with rectal cancer was slightly greater than that observed for colon cancer.

We have previously reported associations for SNPs within subpathways taking into account multiple comparisons. Our goal in these analyses was to summarize the significance of the CHIEF pathway, using hierarchical modeling within ARTP to estimate the overall association as well as to summarize the importance of the subpathways and genes. ARTP utilizes a highly efficient permutation algorithm to determine the significance of association of each gene and of all genes combined, and thus allows us to summarize the statistical significance of the pathway. While many of our results from ARTP are similar to those previously reported, there are some differences. Previously, we focused on assessment of SNPs within genes, adjusting for multiple comparisons and identified SNPs in *FLT1*, *CYP19A1*, *IFNGR1*, *TSC2*, *RUNX3*, *TLR2*, *TLR3*, *TLR4* and selenoproteins that were significant for colon cancer but were not identified here as significant (21–27). For rectal cancer, we identified significant findings for *FLT1*, *VEGFA*, *MAPK8*, *RUNX1*, *SMAD3*, *TLR3*, *STK11* and *PRKAG2* (21,22,24–26,28,29) in our previous work. Differences in association

Table V. Associations for subpathway and gene by tumor molecular phenotype

Subpathway	CIMP			*Kras*			*TP53*			MSI		
	Subpathway P_{ARTP}	Gene	Gene P_{ARTP}	Subpathway P_{ARTP}	Gene	Gene P_{ARTP}	Subpathway P_{ARTP}	Gene	Gene P_{ARTP}	Subpathway P_{ARTP}	Gene	Gene P_{ARTP}
Colon cancer[a]												
Angiogenesis	0.251	*SOD1*	0.017	0.672			0.875			0.104	*MMP7*	0.011
											SOD1	0.016
Hormone/insulin/ growth	0.380			0.169	*EGR2*	0.019	0.069	*PPARG*	0.037	0.156	*IRS1*	0.027
					VDR	0.019		*TCF7L2*	0.003		*PPARG*	0.019
Interferon	0.422	*IRF6*	0.029	0.264	*IRF6*	0.037	0.687			0.812		
Interleukins	0.046	*IL15*	0.032	0.192	*IL17A*	0.031	0.621			0.628		
		IL1A	0.033									
		IL6R	0.027									
		IL8	0.017									
Jak/Stat/Socs	0.234	*SOCS2*	0.015	0.043	*JAK2*	0.041	0.022	*STAT1*	0.023	0.275	*SOCS2*	0.019
					STAT5B	0.014		*STAT5A*	0.008			
					STAT6	0.007		*STAT5B*	0.013			
Pathway core	0.514	*PRKAA1*	0.045	0.555	*PRKAA1*	0.032	0.190	*MTOR*	0.016	0.192	*MTOR*	0.005
		TSC1	0.031					*PRKAA1*	0.009			
MAPK	0.039	*DUSP2*	0.036	0.568			0.640			0.366	*MAP3K9*	0.017
		MAP3K9	0.001									
Selenoprotein	0.751			0.487	*C11orf31*	0.048	0.845			0.680		
Telomere	0.253			0.265			0.149			0.419		
TGFβ	0.181	*BMP4*	0.019	0.027	*BMPR1B*	0.028	0.007	*EIF4EBP3*	0.008	0.194	*BMPR2*	0.039
		RUNX2	0.042		*RUNX2*	0.015		*RUNX3*	0.003		*TGFB1*	0.017
		SMAD2	0.031		*TGFB1*	0.004		*TGFB1*	0.004			
TLR	0.371			0.780			0.509			0.163	*TLR4*	0.041
TNF	0.588			0.322			0.971			0.073	*TNFRSF1A*	0.042
Rectal cancer[b]												
Angiogenesis	0.478			0.849			0.216	*MSTN*	0.042			
								VEGFA	0.014			
Hormone/insulin/ growth	0.781			0.975			0.870					
Interferon	0.655			0.818			0.608					
Interleukin	0.902			0.282	*IL8RB*	0.028	0.024	*IL6*	0.034			
								IL8	0.050			
								IL8RA	0.013			
								IL8RB	0.004			
Jak/Stat/Socs	0.305			0.456			0.561					
Pathway core	0.529	*AKT1*	0.026	0.890			0.590	*NFKB1*	0.050			
		NFKB1	0.041					*PTEN*	0.044			
MAPK	0.794			0.260	*RAF1*	0.030	0.122	*DUSP1*	0.050			
								DUSP6	0.028			
								MAPK8	0.006			
Selenoprotein	0.801			0.066	*SEPN1*	0.008	0.033	*SEP15*	0.029			
					SEPX1	0.043		*TXNRD2*	0.016			
								TXNRD3	0.015			
Telomere	0.134			0.622			0.146					
TGFβ	0.491	*TGFBR1*	0.024	0.956			0.413	*BMP2*	0.021			
TLR	0.210			0.897			0.679					
TNF	0.259			0.763			0.678					

[a]Pathway P_{ARTP} values of 0.324 for CIMP, 0.255 for *Kras*, 0.063 for *TP53* and 0.420 for MSI.
[b]Pathway P_{ARTP} values of 0.843 for CIMP, 0.658 for *Kras* and 0.171 for *TP53*.

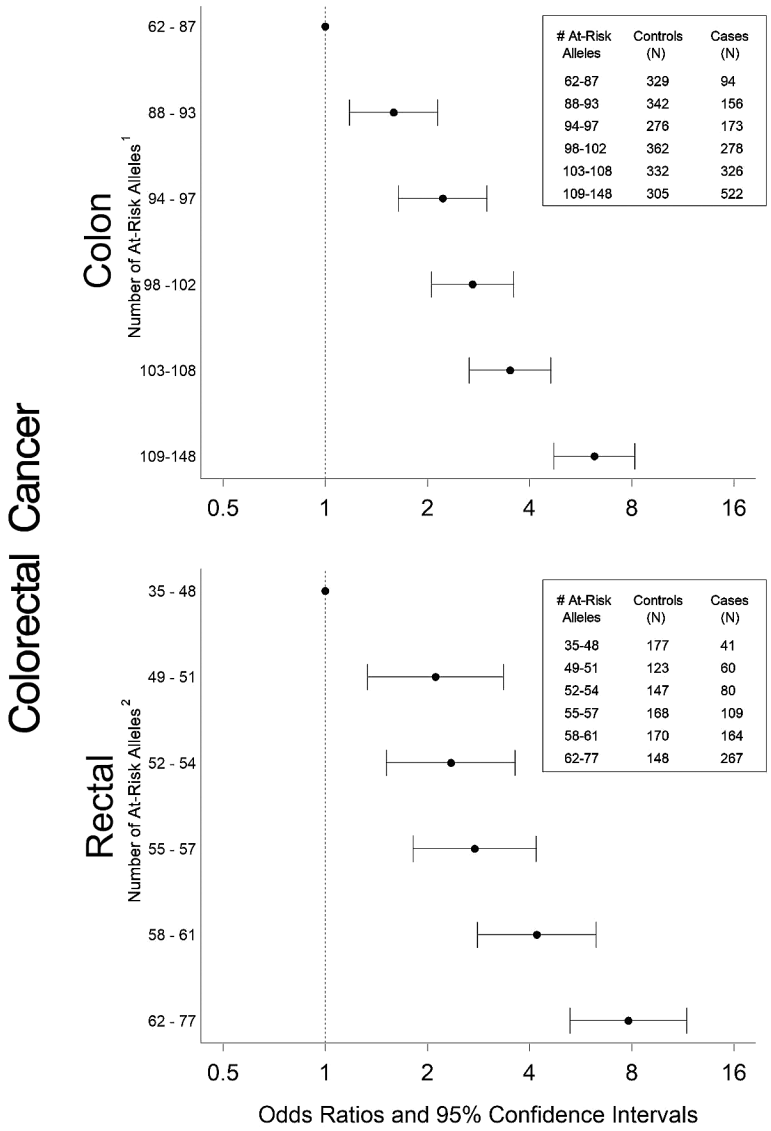

Fig. 2. Polygenic summary score associated with CHIEF pathway for colon and rectal cancer. Models adjusted for age, study center, race/ethnicity and sex. [1]SNPs included in score: *BMP2* rs1979855, rs235770, rs3178250, *BMPR1A* rs6586034, rs7088641, *BMPR1B* rs13134042, rs17616243, rs1863652, rs2120834, rs4490463, rs7662504, rs7694043, rs9307147, *DUSP2* rs1724120, *EIF4E* rs11727086, rs12498533, *FLT1* rs12858139, rs1324057, rs2296189, rs2296283, rs2387632, rs3794400, rs600640, rs678714, rs7324547, rs9513088, *MTOR* rs1057079, rs2024627, *IFNG* rs1861493, rs2069718, rs13117878, rs1519551, *IL2RA* rs12244380, rs12722561, rs12722596, rs3118470, rs706779, *IL3* rs181781, *IL6R* rs4845623, rs7549250, *IL6* rs1800795, rs2069860, *IL8* rs4073, *IRF3* rs2304204, *JAK2* rs1887429, rs3780379, *MAP2K1* rs1432442, rs7181936, rs8039880, *MAP3K3* rs11658329, rs3785574, *MAP3K9* rs11624934, rs11625206, rs11628333, rs11844774, 55 rs17176971, PRKAG2 rs1029947, rs1104897, rs12703162, rs1860743, rs2374270, rs2536068, rs6464156, rs6464170, rs6947064, rs6965771, rs953221, rs9632641, rs9648723, rs9648724, *RPS6KB2* rs917570, *RUNX1* rs2242878, rs2248720, rs2252585, rs2268281, rs2834645, rs2834650, rs2834670, rs7279123, rs8134179, *SLC2A4* rs5435, SMAD2 rs1787199, rs4940086, *SMAD3* rs12901071, rs12904944, rs1498506, rs2414937, *SMAD7* rs12953717, rs4939827, *SOCS2* rs768775, *STAT3* rs1026916, rs12949918, rs6503695, rs8069645, *STAT5A* rs7217728, *STAT5B* rs6503691, rs7218653, *STAT6* rs324011, rs324015, *TCF7L2*, *TERT* rs2736100, rs2736118, rs2853668, *TGFB1* rs1800469, rs4803455, *TGFBR1* rs1571590, rs6478974, *TNF* rs1799964, rs1800630, *VDR*_Fok1, *VDR*_Poly; [2]SNPs included in score: *BMPR2* rs17199235, rs2228545, rs4675278, *DUSP1* rs322351, *DUSP7* rs9851576, *IFNGR1* rs3799488, rs9376267, *IGF1R*, *IL15* rs12508866, rs13117878, rs17461269, *IL8RA* rs1008562, *IL8RB* rs1126579, *IRF2* rs10009261, rs3733473, rs3775554, rs3775556, rs3775574, rs6827018, rs7677486, *MAPK8* rs10508901, *MPO* rs2243828, *NFAT5* rs12447326, rs16959025, *NFKB1* rs11722146, rs230510, rs3821958, *PIK3CA* rs2699905, rs7640662, rs7651265, *PIK3CG* rs11766675, rs4460309, *RPS6KA2* rs1040446, rs10946164, rs1202621, rs2345067, rs4709127, rs6911624, rs7745781, rs9347128, *RPS6KB2* rs1638588, *SEPN1* rs11247735, rs2072749, rs4659382, rs718391, *SEPP1* rs11959466, rs28919882, rs31877899, *STAT3* rs2293152, *STAT6* rs3024979, *TSC1* rs13295634, *TXNRD3* rs11718498, rs4679274, rs9637365, *VEGFA* rs2010963.

stem from the focus on the analysis. Using ARTP, we focus on genes and pathways and only considered SNPs to be important if the gene was statistically significant ($P_{ARTP} < 0.05$ or marginally significant $P_{ARTP} < 0.10$). Previously, we focused on SNPs within genes. While these SNPs may still be important, having only one or two modest associations would not necessarily result in the gene being significant.

However, the major difference in our current and previous findings stems from the inability to incorporate lifestyle exposures and evaluate gene by environment interactions in ARTP. Our previous analyses have shown that multiple lifestyle factors interact with pathway genes, and that the risk associated with genes alone underestimates their influence on CRC risk. Important lifestyle factors that interact with genes in the pathway are dietary components with MAPK (28,30), angiogenesis (31) and Toll-like receptor (TLR) (26) genes; non-steroidal anti-inflammatory drugs use with angiogenesis (31), estrogen-related genes (22), cytokines (32,33), MAPK (28), JAK/STAT (34), TGFβ signaling pathway (29), TLR (26) and selenoproteins (23); cigarette smoking with angiogenesis (31), cytokines

(27,33), MAPK (28), JAK/STAT (34), TGFβ (29) and selenoproteins (23) subpathways; body mass index with angiogenesis (31), estrogen-related genes (22), *VDR* (35) and cytokines (27,33). Thus, to fully estimate the importance of this pathway, methods to evaluate genetic interactions with lifestyle factors are needed.

The polygenic score allowed us to summarize the magnitude of the risk associated with the pathway, rather than merely looking at a *P* value. We determined significance based on the ARTP method and incorporated genes that had a *P* value of <0.10 and SNPs within these genes with a *P* value of <0.10. This was more conservative than including only those genes and SNPs with the smallest *P* values. Increasing risk was observed with increasing number of 'at-risk' genotypes for both colon and rectal cancer.

Based on our findings, several subpathways and groups of genes were significantly associated with colon cancer. The TGFβ signaling pathway appeared to be most important for colon cancer, with *BMP2*, *BMPR1A*, *BMPR1B*, *EIF4E, SMAD2*, *SMAD3*, *SMAD7* and *TGFB1* associated with risk. The TGFβ signaling pathway is an essential regulator of cellular proliferation, differentiation, apoptosis and extracellular matrix remodeling in the cell that is involved in angiogenesis and inflammation (13). It mediates intracellular actions of proinflammatory cytokines, including activation of NF-κB (36,37). BMPs trigger a Smad-signaling cascade that has been linked to reduced cell proliferation and cellular growth (38,39) and may play a key role in regulating tumor initiation. Genome-wide association study have reported that both *BMP2* and *BMP4* were 2 of the top 10 genes identified as contributing to colon cancer risk (40). *BMPR1A* and *BMPR1B* are the two best-characterized type I BMP receptors. Smad proteins are substrates for these receptors and are key intracellular mediators of the transcriptional responses to TGFβ signaling (41). STAT3 has been shown to be a promoter of tumor invasiveness and angiogenesis (42). Activation of STAT5 results in regulation of several genes involved in cell apoptosis, survival and proliferation (43). It has been shown that aspirin, a consistently recognized protective factor for CRC, regulates apoptosis by downregulating the IL6-STAT3 pathway (44).

Along the pathway core, *MTOR* and *RPS6KB2* were significantly associated with colon cancer. *MTOR* represses anabolic processes (ATP utilization) and enhances catabolic processes (ATP generation), restoring the system toward normal energy homeostasis, whereas RPS6KB is involved in a signaling pathway that involves angiotensin II activation of NF-κB (45). The importance of cytokines, including interferons, interleukins and TNF and the MAPK and STATs that are involved in multiple subpathways, appeared to be more important than those genes directly included with hormones, growth factors and insulin-related factors. However, *SLC2A4*, also known as *GLUT4*, was associated with colon cancer. Studies have shown that the cytokine TNF decreases GLUT4 expression in adipocytes resulting in impaired insulin action (46). *VDR* has been associated with colon cancer risk in numerous studies (47,48). Studies have shown that *VDR* interacts with *PPARG* to alter rectal cancer risk (49) and is involved in TGFβ/Smad3 signaling (50). Interleukins are a type of cytokine that control growth and differentiation, cell migration and inflammatory and anti-inflammatory responses by the immune system. TNF, a proinflammatory cytokine, stimulates cell proliferation and induces cell differentiation and is thought to be one of the most important promoters of inflammation. TNF mediates cell survival and apoptosis through TNF receptors by activating at least two major signaling pathways, NF-κB and the p38 MAPK pathway.

A different set of genes was identified for rectal cancer than for colon cancer. Although some of the same subpathways were identified as containing significant genes, those identified as associated with rectal cancer risk were, for the most part, different than those identified for colon cancer. Only four genes, *IL15*, *STAT3*, *STAT6* and *RPS6KB2*, were associated with both colon and rectal cancer and only *IL15* rs13117878 was associated with both cancer sites with the same magnitude of association. For instance, along the pathway core, *NFKB1*, *PIK3CG* and *TSC1* were associated with rectal cancer compared with *MTOR* and *RPS6KB2* that were identified for colon cancer; only *BMPR2* was associated with rectal cancer, whereas eight genes in the TGFβ signaling pathway were associated with colon cancer; *IL8* receptors were associated with rectal cancer, whereas *IL6R* and *IL8* were associated with colon cancer. Selenoproteins appeared to be important for rectal cancer. While differences were observed between colon and rectal cancer, it is important to note that the sample size was considerably less for rectal cancer, and failure to detect associations observed for colon cancer could be from the smaller sample size. Despite few significant genes being associated with rectal cancer, the polygenic score indicated slighter greater risk for rectal cancer. This could indicate that the associations were generally stronger for SNPs that were associated or that the combined effect was greater since the SNPs associated contributed independently. It is possible that having additional at-risk SNPs may not contribute additional risk, given the presence of other SNPs in the pathway.

Evaluation of tumor molecular phenotype showed most similarities between *TP53* and overall colon cancer, which would be expected, given that *TP53* is the most common tumor molecular phenotype in CRCs and therefore of interest. The power to detect associations was weaker for the less common tumor molecular phenotypes such as MSI and CIMP. However, *SOD1*, *SOCS1* and *MAP3K9* were only associated with risk for MSI and CIMP+ tumors, whereas cytokines appeared to be more uniformly associated with CIMP+ tumors. These data illustrate the importance of incorporating tumor molecular phenotype when evaluating risk, given that unique pathways and genes may be associated with specific tumor molecular phenotypes that would be missed if separate analyses were not conducted. Many strengths and limitations have been discussed by Ogino and colleagues (51). Strengths of our study is nearly complete ascertainment of all diagnosed cases of CRC in the target areas (97% for Utah and 85% for Kaiser) which decreases selection bias and increases generalizability to other populations. Likewise, our sample size is large which enables a more powerful assessment of specific tumor molecular phenotype. However, there are some limitations when doing such analysis, including varying sample sizes for the various tumor phenotypes, thus ability to detect associations is not uniform across tumor phenotypes. Additionally, in more traditional analysis, penalties for multiple comparisons could be applied. However, our statistical approach is powerful in that incorporates adjustments as part of the statistical computation. Additionally, error in lab measurements are possible; however, because we did sequencing of *TP53* rather than immunohistochemistry, we have more accurate measurements.

The study had several other strengths and limitations. The pathway approach was novel and an attempt to summarize the statistical significance as well as the related risk with a pathway. ARTP allows us to adaptively combine single SNP *P* values using the rank truncated product statistic and assess significance via permutations at multiple levels, including the gene, subpathway and overall pathway level. However, our results could be from chance and therefore need replication. While we selected genes that we believed were most important to the pathway, there are many other genes and SNPs involved in this pathway that could be important and contribute to colon and rectal cancer risk. Because of our using a customized platform, we were unable to include all potentially relevant genes along the pathway. One of the major limitations is our inability to evaluate the risk associated with these genes from their interaction with lifestyle factors. Our previous analyses suggest that interaction is important, however, at this time, we are only able to determine the significance of these genes based on their main effect and not that component of risk that comes from interaction.

In summary, the CHIEF pathway was significantly associated with colon cancer overall and marginally associated with rectal cancer. However, increasing numbers of at-risk alleles increased risk of both colon and rectal cancer. The TGFβ signaling pathway was the most important subpathway for colon cancer. Other important subpathways were those that included cytokines, JAK/STAT/SOC and MAPK.

Supplementary material

Supplementary Tables 1 and 2 can be found at http://carcin.oxfordjournals.org/

Funding

National Cancer Institute (NCI) (CA48998); The Utah Cancer Registry—funded by contract #N01-PC-67000 from the National Cancer Institute—with additional support: State of Utah Department of Health; Northern California Cancer Registry; Sacramento Tumor Registry.

Acknowledgements

We would like to acknowledge the contributions of Dr Bette Caan, Judy Morse, Donna Schaffer and the Kaiser Permanente Medical Research Program; Sandra Edwards and Jennifer Herrick at the University of Utah and Dr Kristin Anderson and Dr John Potter for data management and collection at the University of Minnesota. The contents of this manuscript are solely the responsibility of the authors and do not necessarily represent the official view of the National Cancer Institute.

Conflict of Interest Statement: None declared.

References

1. Slattery,M.L. *et al*. (2009) Convergence of hormones, inflammation, and energy-related factors: a novel pathway of cancer etiology. *Cancer Prev. Res. (Phila)*., **2**, 922–930.
2. Carling,D. (2004) Ampk. *Curr. Biol*., **14**, R220.
3. Hardie,D.G. (2003) Minireview: the AMP-activated protein kinase cascade: the key sensor of cellular energy status. *Endocrinology*, **144**, 5179–5183.
4. Carling,D. (2004) The AMP-activated protein kinase cascade–a unifying system for energy control. *Trends Biochem. Sci*., **29**, 18–24.
5. Viollet,B. *et al*. (2003) Physiological role of AMP-activated protein kinase (AMPK): insights from knockout mouse models. *Biochem. Soc. Trans*., **31**, 216–219.
6. Watson,C.J. *et al.* (2000) Identification of polymorphisms within the vascular endothelial growth factor (VEGF) gene: correlation with variation in VEGF protein production. *Cytokine*, **12**, 1232–1235.
7. Waldner,M.J. *et al.* (2010) VEGF receptor signaling links inflammation and tumorigenesis in colitis-associated cancer. *J. Exp. Med*., **207**, 2855–2868.
8. Nilsson,B.O. (2007) Modulation of the inflammatory response by estrogens with focus on the endothelium and its interactions with leukocytes. *Inflamm. Res*., **56**, 269–273.
9. McKay,L.I. *et al.* (1999) Molecular control of immune/inflammatory responses: interactions between nuclear factor-kappa B and steroid receptor-signaling pathways. *Endocr. Rev*., **20**, 435–459.
10. De Bosscher,K. *et al.* (2006) Cross-talk between nuclear receptors and nuclear factor kappaB. *Oncogene*, **25**, 6868–6886.
11. Chadwick,C.C. *et al.* (2005) Identification of pathway-selective estrogen receptor ligands that inhibit NF-kappaB transcriptional activity. *Proc. Natl Acad. Sci. USA*., **102**, 2543–2548.
12. Ray,P. *et al.* (1997) Repression of interleukin-6 gene expression by 17 beta-estradiol: inhibition of the DNA-binding activity of the transcription factors NF-IL6 and NF-kappa B by the estrogen receptor. *FEBS Lett*., **409**, 79–85.
13. Gordon,K.J. *et al.* (2008) Role of transforming growth factor-beta superfamily signaling pathways in human disease. *Biochim. Biophys. Acta.*, **1782**, 197–228.
14. Ono,M. (2008) Molecular links between tumor angiogenesis and inflammation: inflammatory stimuli of macrophages and cancer cells as targets for therapeutic strategy. *Cancer Sci*., **99**, 1501–1506.
15. Slattery,M.L. *et al.* (1997) Energy balance and colon cancer–beyond physical activity. *Cancer Res*., **57**, 75–80.
16. Slattery,M.L. *et al.* (2003) Physical activity and colorectal cancer. *Am. J. Epidemiol*., **158**, 214–224.
17. Yu,K. *et al.* (2009) Pathway analysis by adaptive combination of P-values. *Genet. Epidemiol*., **33**, 700–709.
18. Samowitz,W.S. *et al.* (2005) Evaluation of a large, population-based sample supports a CpG island methylator phenotype in colon cancer. *Gastroenterology*, **129**, 837–845.
19. Slattery,M.L. *et al.* (2000) Associations between cigarette smoking, lifestyle factors, and microsatellite instability in colon tumors. *J. Natl. Cancer Inst*., **92**, 1831–1836.
20. Slattery,M.L. *et al.* (2009) A comparison of colon and rectal somatic DNA alterations. *Dis. Colon Rectum*, **52**, 1304–1311.
21. Slattery,M.L. *et al.* (2011) Associations between genetic variation in RUNX1, RUNX2, RUNX3, MAPK1 and eIF4E and risk of colon and rectal cancer: additional support for a TGF-β-signaling pathway. *Carcinogenesis*, **32**, 318–326.
22. Slattery,M.L. *et al.* (2011) Variation in the CYP19A1 gene and risk of colon and rectal cancer. *Cancer Causes Control*, **22**, 955–963.
23. Slattery,M.L. *et al.* (2012) Genetic variation in selenoprotein genes, lifestyle, and risk of colon and rectal cancer. *PLoS One*, **7**, e37312.
24. Slattery,M.L. *et al.* (2014) VEGFA, FLT1, KDR and colorectal cancer: assessment of disease risk, tumor molecular phenotype, and survival. *Mol. Carcinog*., **53** (suppl. 1), E140–E150.
25. Slattery,M.L. *et al.* (2010) Genetic variation in a metabolic signaling pathway and colon and rectal cancer risk: mTOR, PTEN, STK11, RPKAA1, PRKAG2, TSC1, TSC2, PI3K and Akt1. *Carcinogenesis*, **31**, 1604–1611.
26. Slattery,M.L. *et al.* (2012) Toll-like receptor genes and their association with colon and rectal cancer development and prognosis. *Int. J. Cancer*, **130**, 2974–2980.
27. Slattery,M.L. *et al.* (2011) Interferon-signaling pathway: associations with colon and rectal cancer risk and subsequent survival. *Carcinogenesis*, **32**, 1660–1667.
28. Slattery,M.L. *et al.* (2012) MAP kinase genes and colon and rectal cancer. *Carcinogenesis*, **33**, 2398–2408.
29. Slattery,M.L. *et al.* (2012) Genetic variation in the transforming growth factor-β-signaling pathway, lifestyle factors, and risk of colon or rectal cancer. *Dis. Colon Rectum*, **55**, 532–540.
30. Slattery,M.L. *et al.* (2013) Dietary influence on MAPK-signaling pathways and risk of colon and rectal cancer. *Nutr. Cancer*, **65**, 729–738.
31. Slattery,M.L. *et al.* (2012) Oxidative balance and colon and rectal cancer: interaction of lifestyle factors and genes. *Mutat. Res*., **734**, 30–40.
32. Slattery,M.L. *et al.* (2011) Tumor necrosis factor-related genes and colon and rectal cancer. *Int. J. Mol. Epidemiol. Genet*., **2**, 328–338.
33. Bondurant,K.L. *et al.* (2013) Interleukin genes and associations with colon and rectal cancer risk and overall survival. *Int. J. Cancer*, **132**, 905–915.
34. Slattery,M.L. *et al.* (2013) JAK/STAT/SOCS-signaling pathway and colon and rectal cancer. *Mol. Carcinog*., **52**, 155–166.
35. Slattery,M.L. *et al.* (2004) Associations between BMI, energy intake, energy expenditure, VDR genotype and colon and rectal cancers (United States). *Cancer Causes Control*, **15**, 863–872.
36. Hong,S. *et al.* (2007) Smad7 sensitizes tumor necrosis factor induced apoptosis through the inhibition of antiapoptotic gene expression by suppressing activation of the nuclear factor-kappaB pathway. *Cancer Res*., **67**, 9577–9583.
37. Halder,S.K. *et al.* (2005) Smad7 induces tumorigenicity by blocking TGF-beta-induced growth inhibition and apoptosis. *Exp. Cell Res*., **307**, 231–246.
38. Piccirillo,S.G., *et al*. (2006) Bone morphogenetic proteins regulate tumorigenicity in human glioblastoma stem cells. *Ernst Schering Found. Symp. Proc*., **5**, 59–81.
39. Piccirillo,S.G. *et al.* (2006) Bone morphogenetic proteins inhibit the tumorigenic potential of human brain tumour-initiating cells. *Nature*, **444**, 761–765.
40. Peters,U. *et al.* (2012) Meta-analysis of new genome-wide association studies of colorectal cancer risk. *Hum. Genet*., **131**, 217–234.
41. Yang,G. *et al.* (2010) Smad4-mediated TGF-beta signaling in tumorigenesis. *Int. J. Biol. Sci*., **6**, 1–8.
42. Haricharan,S. *et al.* (2014) STAT signaling in mammary gland differentiation, cell survival and tumorigenesis. *Mol. Cell. Endocrinol*., **382**, 560–569.
43. Wei,L. *et al.* (2008) New insights into the roles of Stat5a/b and Stat3 in T cell development and differentiation. *Semin. Cell Dev. Biol*., **19**, 394–400.
44. Kim,S.R. *et al.* (2009) Aspirin induces apoptosis through the blockade of IL-6-STAT3 signaling pathway in human glioblastoma A172 cells. *Biochem. Biophys. Res. Commun*., **387**, 342–347.
45. Zhang,L. *et al.* (2005) A new cellular signaling mechanism for angiotensin II activation of NF-kappaB: an IkappaB-independent, RSK-mediated phosphorylation of p65. *Arterioscler. Thromb. Vasc. Biol*., **25**, 1148–1153.
46. Tesz,G.J. *et al.* (2007) Tumor necrosis factor alpha (TNFalpha) stimulates Map4k4 expression through TNFalpha receptor 1 signaling to c-Jun and activating transcription factor 2. *J. Biol. Chem*., **282**, 19302–19312.
47. Murtaugh,M.A. *et al.* (2006) Vitamin D receptor gene polymorphisms, dietary promotion of insulin resistance, and colon and rectal cancer. *Nutr. Cancer*, **55**, 35–43.
48. Peters,U. *et al.*; Prostate, Lung, Colorectal and Ovarian Cancer Screening Project Team. (2004) Circulating vitamin D metabolites, polymorphism in vitamin D receptor, and colorectal adenoma risk. *Cancer Epidemiol. Biomarkers Prev*., **13**, 546–552.

49. Slattery,M.L. *et al.* (2006) PPARgamma and colon and rectal cancer: associations with specific tumor mutations, aspirin, ibuprofen and insulin-related genes (United States). *Cancer Causes Control*, **17**, 239–249.
50. Daniel,C. *et al.* (2007) The TGFbeta/Smad 3-signaling pathway is involved in butyrate-mediated vitamin D receptor (VDR)-expression. *J. Cell. Biochem*., **102**, 1420–1431.
51. Ogino,S. *et al.* (2011) Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut*, **60**, 397–411.

Received July 2, 2014; revised October 1, 2014; accepted October 8, 2014