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Genetic variation in bone morphogenetic protein (BMP) and colon and rectal cancer

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Abstract

Bone morphogenetic proteins (BMP) are part of the TGF-β-signaling pathway; genetic variation in these genes may be involved in colorectal cancer. In this study we evaluated the association between genetic variation in *BMP1* (11 tagSNPs), *BMP2* (5 tagSNPs), *BMP4* (3 tagSNPs), *BMPR1A* (9 tagSNPs), *BMPR1B* (21 tagSNPs), *BMPR2* (11 tagSNPs), and *GDF10* (7 tagSNPs) with risk of colon and rectal cancer and tumor molecular phenotype. We used data from population-based case-control studies (colon cancer n=1574 cases, 1970 controls; rectal cancer n=791 cases, 999 controls). We observed that genetic variation in *BMPR1A*, *BMPR1B*, *BMPR2*, *BMP2*, and *BMP4* was associated with risk of developing colon cancer, with 20 to 30% increased risk for most high-risk genotypes. A summary of high-risk genotypes showed over a twofold increase in colon cancer risk at the upper risk category (OR 2.49 95% CI 1.95, 3.18). *BMPR2*, *BMPR1B*, *BMP2*, and *GDF10* were associated with rectal cancer. *BMPR2* rs2228545 was associated with an almost twofold increased risk of rectal cancer. The risk associated with the highest category of the summary score for rectal cancer was 2.97 (95% CI 1.87, 4.72). Genes in the BMP-signaling pathway were consistently associated with CIMP+ status in combination with both *KRAS*-mutated and MSI tumors. BMP genes interacted statistically significantly with other genes in the TGF-β-signaling pathway, including *TGFβ1*, *TGFβR1*, *Smad* 3, *Smad* 4, and *Smad* 7. Our data support a role for genetic variation in BMP-related genes in the etiology of colon and rectal cancer. One possible mechanism is via the TGF-β-signaling pathway.

Keywords

bone morphogenetic protein; TGF-β; CIMP+; MSI+; genes; colon cancer; rectal cancer; GDF10

Introduction

The *TGFβ*-signaling pathway plays a critical role in carcinogenesis via regulation of cell growth, differentiation, and proliferation, and apoptosis [1]. As members of the TGFβ-signaling pathway, bone morphogenetic proteins (BMP), may be involved in the initiation

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and progression of colorectal cancer. The BMP pathway has been implicated in the initiation of colorectal cancer among individuals with juvenile polyposis harboring *BMPR1A* receptor mutations [2] Others have shown that the BMP pathway is inactivated in the majority of sporadic colorectal cancer and may be associated with MSI+ tumors [3].

Little is known about the genetic variation in BMP genes and their associations with colon or rectal cancer. However, we know that the TGF-β-signaling pathway, of which BMP is a component, is a key regulatory pathway for colon and rectal cancer. BMPs have been shown to trigger a Smad- signaling cascade that is linked to reduced cell proliferation and cellular growth kinetics of glioblastomas [4, 5] and may play a key role in regulating tumor initiation. A recent genome-wide association study (GWAS) reported that *BMP2* and *BMP4* were two of the top 10 genes identified as associated wtih colon cancer[6]. *BMP4* also has been identified as associated with colorectal cancer in the COGENT Study [7]. Several studies suggest the importance of the BMP receptors, given that BMPs signal through their type I and II receptors [8]. BMPR1A and BMPR1B are the two best characterized type I receptors. Substrates for these receptors include Smad proteins that play a central role in BMP signaling. Genetic variation in *Smad* genes has been associated with colon and rectal cancer [9, 10]. GWAS have shown that *Smad7* is associated with colorectal cancer[6, 7]. Type II BMP receptors, such as BMPR2, like type I receptors, are necessary for BMP signaling.

In this study, we examined genetic variation in *BMP1*, *BMP2*, *BMP4* and their relevant receptor genes *BMPR1A*, *BMPR1B*, *BMPR2*, and Growth Differentiation Factor 10 (*GDF10*) also known as *BMP3B*. We evaluated associations between variants in the BMP pathway with specific tumor markers because others have shown that BMPR2 expression differs by MSI status [3]. Because BMP genes are part of a larger TGF-β-signaling pathway we assessed interaction between BMP genes and other genes in that pathway, including *TGFβ1*, *TGFβR1*, *Smad3*, *Smad4*, *Smad7*, and *NFκB1*.

Methods

Two population-based study populations are included in these analyses. The first study, a population-based case-control study of colon cancer, included cases (n=1,593) and controls (n=1,994) identified between October1, 1991 and September 30, 1994 [11] living in the Twin Cities Metropolitan Area, Kaiser Permanente Medical Care Program of Northern California (KPMCP) and a seven-county area of Utah. The second study, with identical data collection methods, included population-based cases with cancer of the rectosigmoid junction or rectum (n=790) and controls (n=999) who were identified between May 1997 and May 2001 in Utah and KPMCP [12]. Eligible cases were between 30 and 79 years old at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of CRC, and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease.

Controls were matched to cases by sex and by 5-year age groups. At KPMCP, controls were randomly selected from membership lists; in Utah, controls 65 years and older were randomly selected from the Health Care Financing Administration lists and controls younger than 65 years were randomly selected from driver's license lists. In Minnesota, controls were selected from driver's license and state-identification lists. Study details have been previously reported [13, 14].

Interview Data Collection

Data were collected by trained and certified interviewers using laptop computers. All interviews, as previously described, were audio-taped as previously described and reviewed for quality control purposes [15]. The referent period for the study was two years prior to

diagnosis for cases and selection for controls. Detailed information was collected on diet, physical activity, medical history, reproductive history, family history of cancer in first-degree relatives, regular use of aspirin and non-steroidal anti-inflammatory drugs, and body size.

Tumor Marker Data

We have previously evaluated tumors for CpG island methylator phenotype (CIMP), microsatellite instability (MSI), *TP53* mutations, and *KRAS* mutations [16–19] and were therefore able to evaluate BMP-related genes in relation to tumors with specific molecular characteristics. Details of methods used to evaluate epigenetic and genetic changes have been described [16–19]. Given the rarity of MSI+ rectal tumors [20] we were unable to evaluate that small subset of tumors.

TagSNP Selection and Genotyping

TagSNPs were selected for *BMP1*(rs3924229, rs1357482, rs4076873, rs7592, rs7812993, rs4872360, rs12114940, rs3924231, rs4075478, rs3857979, rs11775186), *BMP2 (*rs235770, rs1979855, rs7270163, rs1005464, rs3178250), BMP4 (rs17563, rs762642, rs2761887), *BMPR1A* (rs10887668, rs7895217, rs4934275, rs6586034, rs7088641, rs21687668, rs12765929, rs12415784, rs2883420), BMPR1B (rs7698964, rs7694043, rs7661049, rs1863652, rs9307147, rs11947569, rs13134042, rs6849425, rs4145993, rs7662504, rs12508087, rs3821968, rs6499673, rs4490463, rs10049681, rs2214395, rs2719176, rs17616243, rs17022671, rs2120834, rs3796442), *BMPR2* (rs12477602, rs2350809, rs6751210, rs13430786, rs1980153, rs4303700, rs4675278, rs12621870, rs1199496, rs17199235, rs2228545), *and GDF10* (rs762454, rs2853838, rs7093975, rs1198444, rs12769499, rs1902725, rs1902724) using the following parameters: LD blocks using a Caucasian LD map and an r^2=0.8 defined; minor allele frequency (MAF) >0.1; range= −1500 bps from the initiation codon to +1500 bps from the termination codon; and 1 SNP/LD bin. All markers were genotyped using a multiplexed bead-array assay format based on GoldenGate chemistry (Illumina, San Diego, California). A genotyping call rate of 99.85% was attained. Blinded internal replicates represented 4.4% of the sample set; the duplicate concordance rate was 100%. A detailed summary of these SNPs is available in the online supplement. Genotyping of other genes in the candidate pathway, including *NFκB1*, *TGFβ1*, *TGFβR1*, *Smad3*, *Smad4*, and *Smad7*, which were assessed for their interactive effects with *BMP* genes, were genotyped on the same platform. Individuals with missing genotype data were not included in the analysis for that specific marker.

Statistical Methods

Statistical analyses were performed using SAS® version 9.2 (SAS Institute, Cary, NC). We report odds ratios (ORs) and 95% confidence intervals (95%CIs) assessed from adjusted multiple logistic regression models. TagSNP selection was based on those tagSNPs identified as being statistically significant using multiple logistic regression models adjusting for age, center, race/ethnicity, and sex. To summarize risk associated with multiple variants across the pathway we created a summary score that was based on all at-risk genotypes identified from multiple regression models for colon and rectal cancer. The score for each SNP was based on the inheritance model and its associated risk. For the co-dominant or additive model a score of zero, one, or two was assigned directly related to the number of high-risk alleles, while scores of zero or two were assigned for the dominant and recessive models. After assigning a score for each SNP, the scores were summed across SNPs to generate an individual summary score. The score variable was categorized based on the frequency distribution within the study population. *P* values for trend were determined by comparing a full model including the continuous score term to a score reduced model via a likelihood-ratio test.

Analysis for interaction was based on tagSNPs within each BMP gene with a Wald p value of <0.15 from the initial logistic regression analysis. These SNPs were compared to targeted candidate SNPs within genes in the proposed pathway that were previously identified as being statistically significantly associated with colon and rectal cancer at the 0.05 level or less. Genes tested for interaction were: *TGFβ1* (2 SNPs for colon and rectal cancer), *TGFβR1* (1 SNP for colon cancer only), *Smad2* (2 SNPs for colon and 1 SNP for rectal cancer), *Smad3* (4 SNPs for colon cancer and 1 SNP for rectal cancer), *Smad7* (3 candidate SNPs for both colon and rectal cancer), and *NFκB1* (5 SNPs for both colon and rectal cancer). BMP genes evaluated were *BMP1* (1 SNP for rectal cancer), *BMP2* (3 SNPs for colon cancer and 2 SNPs for rectal cancer), *BMP4* (1 SNP for colon cancer), *BMPR2 (*2 SNPs for both colon and rectal cancer), *BMPR1A* (5 SNPs for colon cancer), *BMPR1B* (10 SNPs for colon cancer and 4 SNPs for rectal cancer), *and GDF10 (*1 SNP for rectal cancer).

Possible interactions between BMP genes and three hypothesized non-gene exposures associated with inflammation (i.e. recent aspirin or NSAID use), estrogen (i.e. recent estrogen use), and insulin (i.e. BMI of <25, 25–30, >30) were evaluated. We believe that inflammation, estrogen, and insulin are central to colon and rectal cancer etiology; these variables were selected as indicators of these lifestyle exposures that may interact with this candidate pathway. *P* values for interaction for genetic and lifestyle factors were determined using a likelihood-ratio test comparing a full model that included an interaction term with a reduced model without an interaction term.

Tumors were defined by specific somatic alterations; any *TP53* mutation; any *KRAS* mutation; MSI+; CIMP+ defined as at least two of five markers methylated; a combination of CIMP+/*KRAS*-mutated; a combination of CIMP+/MSI+. As the proportion of MSI+ tumors in the rectal cases was <3% [20], we did not examine that molecular phenotype in our rectal data. Population-based controls were used to assess associations between tagSNPs in candidate genes and specific tumor molecular phenotypes using the summary score methods described above. Comparisons of cases with and without specific epigenetic and genetic changes were conducted to test for heterogeneity with specific tumor molecular phenotype. The heterogeneity *p* values are based on the likelihood-ratio test comparing a full model with a reduced model excluding the score term, both of which are adjusted for other tumor markers.

Adjusted multiple-comparison p values, taking into account tagSNPs within the gene, were estimated using the methods by Conneely and Boehnke[21] via R version 2.11.0 (R Foundation for Statistical Computing, Vienna, Austria). Wald p values from the original models and interaction p values based on likelihood-ratio tests were used for estimates of multiple comparisons. We consider a pACT of <0.15 as being potentially important given the candidate pathway approach and the need to consider both type 1 and type 2 errors. We believe that findings at this level would merit replication.

Results

The study population is described in Table 1. The majority of participants were white non-Hispanic, male, and over 60 years of age. Table 2 describes the tagSNPs for the candidate genes carried forward into further analyses based on statistically significantly associations with colon and rectal cancer, either overall or with specific tumor markers. All tagSNPs were in HWE. Supplemental Table 1 provides a list of detailed information on all tagSNPs for these genes included on the platform.

Associations between tagSNPs, and risk of colon and rectal cancer are shown in Table 3. For several genes, more than one tagSNP was independently associated with colon cancer;

BMPR1B rs13134042, rs2120834, rs17616243, rs2719176, and rs1863652 were all associated with colon cancer with ORs of similar magnitudes of risk. Seven SNPs had pACT of <0.15 for colon cancer and three SNPs, *BMP2* rs12979855, rs3178250, and *BMPR1A* rs2883420 had pACT values of <0.05. For rectal cancer seven independent tagSNPs were associated with disease risk, representing four genes, *BMP2*, *BMPR1B*, *BMPR2*, and *GDF10*. Of these, *BMPR2* rs17199235 had an adjusted pACT of <0.05 and *BMPR2* rs228545 and *GDF10* rs762454 had pACT values of <0.15. For both colon and rectal cancer, the summary score across tagSNPs showed a significant linear trend of increasing risk associated with increasing number of higher risk genotypes.

Assessment of interaction between BMP-related genes and other genes in the candidate pathway that were hypothesized as interacting with BMP genes, showed several statistically significant interactions. For colon cancer *BMP2* interacted with *NFκB1*, *Smad3*, *TGFβ1*, *Smad2* and *Smad7; BMP4* interacted statistically with *NFκB1 and Smad3*, *BMPR1B* with *NFκB1*, *Smad2*, *Smad7*, *Smad3*, *Smad4*, and *TGFβ1; BMPR1A* with *Smad7* and *TGFβ*1, and *BMPR2* interacted significantly with *Smad3*, *Smad7*, and *TGFβ1*. For rectal cancer *BMP1interacted* with *NFκB1*, *Smad7*, and *TGFβ1; BMP2* interacted statistically significantly with *TGFβ1; BMPR1B* interacted with *Smad7* and *TGFβR1; BMPR2* interacted with *NFκB1* and *TGFβ1*; and *GDF10* interacted with *NFκB1*, *Smad2*, and *TGFβ1* (Table 4). Of the 357 SNPs evaluated (21BMP SNPs with Wald p <0.15 and 17 gene pathway SNPS) for interaction in colon cancer, 62 had a p value of <0.05, of which 37 had a pACT of <0.15 and 11 had pACT values of <0.05. For rectal cancer, we tested 120 SNP interactions (10 BMP SNPS with 12 gene pathway SNPS), of which 23 were significant at the 0.05 level; after adjustment of these SNPs for multiple comparisons, 19 had a pACT at the 0.15 level and 7 had a pACT at the 0.05 level.

We evaluated the combined effects of the *BMP* genes with various molecularly defined colon and rectal tumor phenotypes (Table 5). The colon tumor phenotypes most influenced by the *BMP* genes were CIMP+, MSI+, *KRAS*-mutated, and combinations of these epigenetic and genetic molecular changes. *KRAS*-mutated tumors were not associated with BMP-related genes for rectal cancer, although *TP53*-mutated tumors were. Risk summary scores showed increasing risk with increasing number of at-risk genotypes for both colon and rectal cancer. The p value for heterogeneity indicates that the majority of associations were unique to the specific tumor molecular phenotype. The magnitude of the associations with tumor markers was slightly stronger for rectal tumors than for colon tumors.

There were few statistically significant interactions between BMP genes and obesity and recent use of aspirin/NSAIDS or estrogen status (data not shown in table). *BMP2* rs235770 interacted statistically significantly with BMI; those with the TT genotype had a greater risk of rectal cancer if they had a BMI of 30 or more (OR 2.08 95% CI 1.13,3.82 compared to OR of 0.73 95% CI 0.44,1.24 for normal weight and TT genotype; p interaction 0.0098; pACT 0.02). *BMPR1B* rs9307147 interacted statistically significantly with aspirin/NSAIDs; having the GG genotype reduced colon cancer risk among those without recent use (OR 0.63 95% CI 0.49,0.80 while the GG genotype among aspirin/NSAID users was 1.0; p interaction 0.0288; pACT 0.22). No other meaningful interactions were detected.

Discussion

This study highlights the potential importance of the *BMP* genes in colon and rectal carcinogenesis. Both independently and compositely, these genes are associated with cancer risk. Our findings corroborate the hypothesis that type I and type II receptors of *BMP* genes play a significant role in disease risk. Given the interaction with many other genes within the TGF-β-signaling pathway, it is probable that at least part of their influence in disease risk is

through this signaling pathway and that the pathway may operate through CIMP-related mechanisms in combination with *KRAS*-mutated tumors and MSI+ tumors.

Loss of BMP signaling has been shown to be highly prevalent in sporadic colon cancers [22]. BMP acts as a tumor suppressor that is involved in apoptosis; disturbances in BMP signaling could lead to tumorigenesis [23]. BMP also is a member of the TGF-β superfamily that plays a critical role in colorectal cancer. BMP signaling is mediated by its receptors and their downstream molecules such as Smad. Approximately 50% of individuals with juvenile polyposis carry germline mutations in either *BMPR1A* or *Smad4* genes [24]. Thus, there is a clear biologically plausible role for BMP genes in the etiology of colorectal cancer.

An important consideration when determining risk associated with genes hypothesized as being a component of a candidate pathway is how they work together as well as independently. It is generally unknown if having one or multiple SNPs have similar effects on risk. For instance, does the risk increase with the number of high-risk genotypes or do multiple high-risk genotypes have a minimal effect beyond any individual high-risk genotype in the candidate pathway? For both colon and rectal cancer, it appears that having multiple high-risk genotypes increases the risk of cancer. The summary risk appeared to have slightly greater effect for rectal cancer than for colon cancer. Our findings illustrate the importance of assessing multiple candidate genes together to obtain a better understanding of their relevance to the overall pathway.

In addition to evaluating how *BMP* genes work together, we evaluated how these work as part of the TGF-β-signaling pathway. We observed statistically significant interactions with several genes within this pathway, including *TGFβ1*, *TGFβR1*, *Smad3*, *Smad4*, and *Smad7*. The statistically significant interaction observed between BMP-related genes and other genes within the TGF-β-signaling pathway supports the concept that multiple components in the pathway influence disease risk, not just isolated genes or SNPs. Additionally, the combined effects of variation in genes within the TGF-β-signaling pathway on colon and rectal cancer risk provides additional support for the importance of this pathway in colon and rectal cancer.

Others have reported that *BMPR2* is associated with MSI+ tumors [25]. Our data suggest that in addition to associations with MSI, BMP-related genes are associated with CIMP+ tumors. Statistically significant associations were observed for CIMP+ tumors in combination with both MSI+ tumors and *KRAS*- mutated tumors. Our previous report on polymorphism in *TGFβ1*, *TGFβR1*, and *Smad* genes [26] also suggested that CIMP+ tumors were highly associated with these genes. These data add to the evidence that the TGF-β-signaling pathway is important in the etiology of CIMP+ tumors.

This study was hypothesis driven, assessing candidate genes along a biologically defined candidate pathway. The genes were selected because of their biologic function and potential importance in the regulation of the TGF-β-signaling pathway. Because little is known about these genes, including which SNPs are functional, we used a tagSNP approach to characterize genetic variation within the gene that may influence disease risk. The Cogent GWAS Study reported significant associations for *BMP4* rs4444235[7]. A small subset of our data have this tagSNP available and we observed a non-significant risk estimate of 1.18 (95% CI 0.88,1.57) for this SNP, which is comparable to the significant risk estimate of 1.12 (95% CI 1.07–1.18) reported for rs4444235 in the Cogent Study. Although we identified several *BMP4* SNPs that were associated with colon cancer they had low D′ values compared to this previously reported SNP; *BMP4* rs762642 with a D′ of 0.447 was the only BMP SNP with a value of greater than 0.08 compared to rs444235. TagSNPs, although not necessarily functional, serve as an indication that variation in a relevant gene contributes to

disease risk. The identification of functional SNPs in linkage with the tagSNPs is outside the scope of this report, but identification of functional SNPs within these genes could potentially contribute to both improved risk assessment and the development of targeted therapies

Our analysis plan included many comparisons that were necessary to consolidate the data into a more coherent picture of how BMP-related genes are associated with colon and rectal cancer. To address how tagSNPs operated together we calculated summary scores across high-risk genotypes as defined from our initial analysis. Given our limited information on these genes prior to our analysis such selection seems justified. We used the pACT to give an indication of the potential importance of statistically significant individual tagSNPs considering the comparisons being made. We report Wald and likelihood ratio p values that were used to adjust for multiple comparisons. The pACT and other methods should be viewed as an indication of risk of false positive results taking into account the comparisons being made. However, it is important for other studies to replicate these results and conduct experiments to test the functionality of potentially important SNPs and genes, thus we considered a pACT of <0.15 as potentially meaningful for replication purposes and to avoid type 2 errors. These results need confirmation in other large studies of colon and rectal cancer, particularly the question of whether variation in BMP-related genes do, indeed, act in concert to cumulatively elevate risk and if there is a similar pattern of cumulative risk with other members of the TGF-β-signaling pathway

Few studies have examined BMP-related genes and risk of colon and rectal cancer despite the biologic plausibility for an association. Their importance is potentially highlighted by GWAS that have identified both *BMP2* and *BMP4* among top 10 hits with colon cancer. Here we report that in addition to confirming the role of *BMP2* and *BMP4* in colon and rectal cancer etiology, we show that other BMP genes also contribute to both colon and rectal cancer risk. Our data support the role of BMP genes as an important component of the TGF-β-signaling pathway and further suggest that this pathway may act to elevate risk of CIMP+ colorectal cancer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Description of Study Population

		Colon		Rectal	
		Control	**Case**	**Control**	**Case**
		n (%)	**n (%)**	**n (%)**	**n (%)**
Center	Utah	378 (19.33)	249 (16.01)	365 (38.06)	274 (36.34)
	Kaiser, CA	787 (40.24)	744 (47.85)	594 (61.94)	480 (63.66)
	Minnesota	791 (40.44)	562 (36.14)	NA	NA
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)
Race/Ethnicity	NHW	1828 (93.46)	1428 (91.83)	824 (85.92)	625 (82.89)
	Hispanics	75 (3.83)	59 (3.79)	63 (6.57)	61 (8.09)
	Black	53 (2.71)	68 (4.37)	43 (4.48)	29 (3.85)
	Asian			29 (3.02)	39 (5.17)
Gender	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)

Table 2

Descriptive table of tagSNPs associated with colon and rectal cancer.

Symbol	Location	SNP	Major/Minor Allele	NHW	MAF[1] Hisp	AA	FDR[2] HWE
BMP2	20p12	rs235770	C/T	0.38	0.33	1.00	1.00
		rs1979855	T/C	0.17	0.11	0.16	0.69
		rs3178250	T/C	0.2	0.18	0.23	0.97
BMP4	14q22-q23	rs17563	C/T	0.44	0.40*	0.21*	0.75
BMPR1A	10q22.3	rs6586034	T/G	0.44	0.49	0.23*	0.91
		rs7088641	T/C	0.31	0.35	0.07	0.67
		rs2883420	T/C	0.39	0.43	0.22*	0.96
BMPR1B	4q22-q24	rs7694043	C/T	0.35	0.26	0.12	1.00
		rs1863652	C/T	0.35	0.41	0.23	0.91
		rs9307147	A/G	0.45	0.38	0.24	1.00
		rs11947569	T/C	0.21	0.19	0.22	1.00
		rs13134042	G/A	0.21	0.23	0.08	0.74
		rs6849425	C/T	0.21	0.23	0.19	0.96
		rs7662504	A/C	0.39	0.48*	0.48	1.00
		rs4490463	A/G	0.42	0.34	0.45*	1.00
		rs2719176	C/G	0.38	0.4	0.49*	1.00
		rs17616243	C/T	0.15	0.18	0.07	1.00
		rs2120834	G/C	0.39	0.36	0.47	0.75
BMPR2	2q33-q34	rs6751210	A/G	0.49	0.47	0.44*	0.85
		rs17199235	A/G	0.11	0.04	0.04	0.67
		rs2228545	G/A	0.03	<.01	0.02	1.00
GDF10	10q11.22	rs762454	A/G	0.33	0.29	0.34	1.00

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

* Indicates major/minor allele differs for Hispanic (Hisp) or African American (AA) from non-Hispanic white (NHW)

[2] FDR (HWE) = False Discovery Rate Hardy Weinberg Equilibrium test.

Table 3

Associations between BMP genes and colon and rectal cancer

Colon Cancer		Controls	Cases	OR (95% CI)	P value[2]	pACT[3]
BMP2	rs1979855[4]				0.00056	0.0027
	TT	1381	1014	1.00		
	TC/CC	575	541	1.29 (1.11,1.48)		
	rs3178250				0.0094	0.0359
	TT	1256	931	1.00		
	TC/CC	700	624	1.20 (1.05,1.38)		
	rs235770				0.0223	0.063
	CC	796	691	1.00		
	CT	884	680	0.91 (0.78,1.05)		
	TT	276	184	0.78 (0.63,0.97)		
BMP4	rs17563				0.0315	0.0791
	CC/CT	1567	1191	1.00		
	TT	387	364	1.20 (1.02,1.41)		
BMPR1A	rs2883420				0.0065	0.0492
	TT	685	584	1.00		
	TC/CC	1271	971	0.87 (0.76,1.00)		
	rs7088641[5]				0.0098	0.0666
	TT	904	793	1.00		
	TC/CC	1052	762	0.83 (0.73,0.95)		
	rs6586034				0.0487	0.2592
	TT	555	497	1.00		
	TG/GG	1388	1051	0.82 (0.71,0.95)		
BMPR1B	rs9307147				0.0028	0.0538
	AA	604	548	1.00		
	AG	954	740	0.86 (0.74,1.00)		
	GG	398	267	0.75 (0.62,0.91)		
	rs17616243				0.0094	0.1561
	CC	1427	1077	1.00		

Colon Cancer		Controls	Cases	OR (95% CI)	P value[2]	pACT[3]
	CT/TT	529	478	1.22 (1.05,1.41)		
	rs7662504				0.0169	0.2497
	AA	706	605	1.00		
	AC	931	729	0.90 (0.78,1.04)		
	CC	318	221	0.78 (0.64,0.96)		
	rs4490463				0.0375	0.4134
	AA	646	562	1.00		
	AG	964	746	0.89 (0.76,1.03)		
	GG	345	246	0.82 (0.67,1.00)		
	rs2719176				0.0439	0.428
	CC/CG	1673	1291	1.00		
	GG	283	264	1.21 (1.01,1.45)		
	rs13134042				0.0400	0.4213
	GG/GA	1856	1498	1.00		
	AA	99	57	0.70 (0.50,0.98)		
	rs1863652				0.0299	0.3634
	CC/CT	1711	1398	1.00		
	TT	243	157	0.79 (0.64,0.98)		
	rs2120834				0.0189	0.2647
	GG/GC	1648	1354	1.00		
	CC	307	201	0.79 (0.65,0.96)		
BMPR2	rs6751210				0.0366	0.2816
	AA/AG	1501	1145	1.00		
	GG	455	410	1.18 (1.01,1.38)		
Summary Score						
	(1 – 11)	414	194	1.00		
	(12 – 14)	483	368	1.60 (1.28,1.99)		
	(15 – 17)	477	400	1.76 (1.42,2.19)		
	(18 – 20)	339	312	1.94 (1.54,2.44)		
	(21 – 30)	243	281	2.49 (1.95,3.18)		
	P Trend	<.0001				

Colon Cancer		**Controls**	**Cases**	**OR (95% CI)**	**P value**[2]	**pACT**[3]
Rectal Cancer						
BMP2	rs3178250				0.0403	0.1738
	TT/TC	926	711	1.00		
	CC	33	42	1.63 (1.02,2.60)		
BMPR1B	rs7694043				0.02607	0.3656
	CC	413	367	1.00		
	CT/TT	546	387	0.82 (0.67,1.00)		
	rs6849425				0.042	0.4945
	CC/CT	902	725	1.00		
	TT	57	29	0.62 (0.39,0.98)		
	rs11947569				0.0459	0.5057
	TT/TC	924	710	1.00		
	CC	35	44	1.68 (1.06,2.65)		
BMPR2	rs17199235				0.0016	0.0162
	AA	798	596	1.00		
	AG/GG	161	158	1.35 (1.05,1.73)		
	rs2228545				0.0174	0.1349
	GG	917	695	1.00		
	GA/AA	42	59	1.93 (1.28,2.91)		
GDF10	rs762454				0.0218	0.1068
	AA/AG	862	651	1.00		
	GG	97	102	1.42 (1.05,1.91)		
Summary Score						
	(0 – 2)	402	244	1.00		
	(3 – 4)	394	324	1.32 (1.07,1.65)		
	(5 – 6)	131	129	1.61 (1.20,2.16)		
	(7 – 11)	32	57	2.97 (1.87,4.72)		
	P Trend	<.0001				

[1] Odds Ratio (OR) and 95% Confidence Interval (CI) from multiple logistic regression analysis adjusting for age, sex, center, and race/ethnicity

[2] Wald p value

Int J Cancer. Author manuscript; available in PMC 2013 February 1.

[3] pACT from methods of Conneely and Boehnke[21]

[4] *BMP2* rs1979855 and rs3178250 r^2=0.59

[5] *BMPRIA* rs7088641 and rs6586034 r^2 = 0.58

Table 4

Associations between *BMP* genes and candidate genes in hypothesized pathway

BMP Gene	**SNP (high-risk genotype)**	***Pathway Gene***	**SNP (high- risk genotype)**	**Interaction p value[1]**	**pACT[2]**
Colon Cancer					
BMP2	rs1979855 (TC/CC)	*NFκB1*	rs3821958 (GG)	0.0339	0.1618
		SMAD3	rs12901071 (AA)	0.0065	**0.0309**
		TGFβ1	rs1800469 (GG)	0.0080	**0.0357**
			rs4803455 (AA)	0.0406	0.0907
	rs235770 (CC)	*SMAD2*	rs1787199 (TT)	0.0327	0.0978
		SMAD3	rs2414937 (GG/GC)	0.0015	**0.0082**
			rs7163381 (GG/GA)	0.0325	0.1226
		SMAD7	rs12953717 (TT)	0.0335	0.1397
			rs4939827 (TT)	0.0098	0.0509
		TGFβ1	rs1800469 (GG)	0.0093	**0.0363**
			rs4803455 (AA)	0.0103	**0.0343**
	rs3178250 (TC/CC)	*SMAD2*	rs4940086 (CC)	0.0307	0.0984
		SMAD3	rs12901071 (AA)	0.0126	0.0553
BMP4	rs17563 (TT)		rs12901071 (AA)	0.0024	**0.0051**
		NFκB1	rs230510 (AA)	0.0052	**0.0136**
			rs3821958 (GG)	0.0219	**0.0409**
BMPR1A	rs2168730 (GG)	*SMAD7*	rs4939827 (TT)	0.0015	**0.0153**
	rs2883420 (TT)		rs4464148 (CC)	0.0478	0.2620
	rs7895217 (AA)		rs12953717 (TT)	0.0204	0.1377
			rs4464148 (CC)	0.0108	0.0816
		TGFβ1	rs1800469 (GG)	0.0214	0.1339
BMPR1B	rs13134042 (GG/GA)	*NFκB1*	rs4648110 (TT/TA)	0.0226	0.3525
		SMAD2	rs1787199 (TT)	0.0140	0.1347
		SMAD7	rs12953717 (TT)	0.0058	0.0995
			rs4939827 (TT)	0.0033	0.0627
	rs1863652 (CC/CT)	*NFκB1*	rs11722146 (AA)	0.0449	0.5433
			rs230510 (AA)	0.0193	0.3187
			rs3821958 (GG)	0.0228	0.3526

BMP Gene	SNP (high-risk genotype)	*Pathway Gene*	SNP (high- risk genotype)	Interaction p value[1]	pACT[2]
		SMAD3	rs1498506 (AA)	0.0364	0.4523
		SMAD7	rs12953717 (TT)	0.0192	0.2498
			rs4464148 (CC)	0.0469	0.4390
	rs2120834 (GG/GC)	*SMAD2*	rs1787199 (TT)	0.0404	0.3096
		TGFβ1	rs1800469 (GG)	0.0084	0.1128
			rs4803455 (AA)	0.0070	0.1023
	rs2719176 (GG)	*NFκB1*	rs230510 (AA)	0.0032	0.0776
		SMAD3	rs1498506 (AA)	0.0293	0.3923
	rs3821968 (TT)	*NFκB1*	rs11722146 (AA)	0.0407	0.5180
	rs4490463 (AA)		rs11722146 (AA)	0.0062	0.1322
			rs230510 (AA)	0.0045	0.1016
			rs3821958 (GG)	0.0089	0.1775
		SMAD2	rs1787199 (TT)	0.0497	0.3550
			rs4940086 (CC)	0.0292	0.2431
		SMAD3	rs12901071 (AA)	0.0232	0.3338
			rs1498506 (AA)	0.0045	0.0872
		TGFβ1	rs1800469 (GG)	0.0230	0.2477
	rs4699673 (GG)	*SMAD7*	rs12953717 (TT)	0.0194	0.2467
			rs4939827 (TT)	0.0096	0.1501
	rs7662504 (AA)	*NFκB1*	rs4648110 (TT/TA)	0.0201	0.3245
		SMAD2	rs1787199 (TT)	0.0001	**0.0011**
			rs4940086 (CC)	0.0010	**0.0129**
		SMAD3	rs12901071 (AA)	0.0494	0.5470
		SMAD7	rs12953717 (TT)	0.0125	0.1796
			rs4464148 (CC)	0.0384	0.4035
			rs4939827 (TT)	0.0044	0.0808
	rs9307147 (AA)	*SMAD2*	rs4940086 (CC)	0.0118	0.1186
		SMAD3	rs1498506 (AA)	0.0106	0.1809
		SMAD7	rs4464148 (CC)	0.0206	0.2555
BMPR2	rs1980153 (AA)	*SMAD3*	rs1498506 (AA)	0.0422	0.1452
		SMAD7	rs12953717 (TT)	0.0183	0.0761

BMP Gene	**SNP (high-risk genotype)**	***Pathway Gene***	**SNP (high- risk genotype)**	**Interaction p value[1]**	**pACT[2]**
			rs4464148 (CC)	0.0332	0.0979
			rs4939827 (TT)	0.0261	0.0956
	rs6751210 (GG)	*TGFβ1*	rs4803455 (AA)	0.0172	0.0593
Rectal Cancer					
BMP1	rs3924229 (CC)	*NFκB1*	rs11722146 (AA)	0.0461	0.1251
			rs3821958 (GG)	0.0076	**0.0289**
			rs4648110 (AA)	0.0418	0.1249
		SMAD7	rs12953717 (CC)	0.0238	0.0617
			rs4464148 (TT)	0.0320	0.0624
		TGFβ1	rs1800469 (GG)	0.0241	0.0598
BMP2	rs1979855 (CC)		rs4803455 (AA)	0.0290	0.0561
	rs3178250 (CC)		rs4803455 (AA)	0.0077	**0.0270**
BMPR1B	rs11947569 (CC)	*SMAD7*	rs12953717 (CC)	0.0185	0.1263
			rs4939827 (TT)	0.0257	0.1580
	rs13134042 (GA/AA)	*NFκB1*	rs230510 (AA)	0.0404	0.3058
			rs4648110 (AA)	0.0196	0.1703
		SMAD7	rs12953717 (CC)	0.0356	0.1907
	rs6849425 (CC/CT)	*NFκB1*	rs3821958 (GG)	0.0135	0.1300
		SMAD7	rs4464148 (TT)	0.0053	**0.0463**
			rs4939827 (TT)	0.0166	0.1224
BMPR2	rs17199235 (AG/GG)	*TGFβ1*	rs1800469 (GG)	0.0017	**0.0063**
	rs2228545 (GA/AA)	*NFκB1*	rs230510 (AA)	0.0213	0.0960
			rs3821958 (GG)	0.0067	**0.0360**
		TGFβ1	rs1800469 (GG)	0.0344	0.0903
GDF10	rs762454 (GG)	*NFκB1*	rs4648110 (AA)	0.0492	0.1216
		SMAD2	rs1792689 (CC)	0.0206	**0.0206**
		TGFβ1	rs4803455 (AA)	0.0086	**0.0165**

[1] likelihood ratio p value

[2] pACT from methods of Conneely and Boehnke [21]

Table 5

Associations between colon and rectal tumor molecular phenotype and BMP genes

Score[1]	N			OR (95%CI)[2]	OR (95% CI)[2]	Heterogeneity
	Controls	Case+	Cases−	Case+ vs Controls CIMP+	CASE+ vs. CASE−	p value[3]
Colon Cancer						
(0 – 3)	488	43	179	1.00	1.00	
(4 – 6)	968	137	368	1.57 (1.10,2.26)	1.46 (0.94,2.26)	0.0869
(7 – 8)	425	75	162	2.04 (1.37,3.04)	2.07 (1.25,3.42)	0.0040
(9 – 10)	75	17	29	2.60 (1.40,4.82)	1.99 (0.89,4.44)	0.1000
				KRAS		
(0 – 4)	492	52	183	1.00	1.00	
(5 – 6)	561	101	203	1.63 (1.14,2.33)	1.70 (1.09,2.64)	0.0178
(7 – 8)	441	92	162	1.94 (1.34,2.79)	2.09 (1.33,3.30)	0.0013
(9 – 12)	462	103	195	2.05 (1.44,2.94)	1.70 (1.08,2.68)	0.0208
				MSI+		
(0 – 6)	451	23	218	1.00	1.00	
(7 – 8)	323	24	156	1.43 (0.79,2.58)	1.92 (0.81,4.58)	0.1382
(9 – 11)	567	52	313	1.80 (1.08,2.98)	1.34 (0.68,2.66)	0.3992
(12 – 14)	435	49	219	2.15 (1.28,3.60)	2.47 (1.25,4.88)	0.0078
(15 – 18)	180	37	84	3.98 (2.29,6.89)	4.27 (1.86,9.80)	0.0004
				CIMP+& *KRAS*		
(0 – 2)	1073	27	243	1.00	1.00	
(4 – 4)	704	31	171	1.71 (1.01,2.90)	1.71 (0.96,3.04)	0.0680
(6 – 6)	179	16	36	3.58 (1.88,6.80)	3.80 (1.73,8.38)	0.0013
				CIMP+ & MSI+		
(0 – 6)	592	15	196	1.00	1.00	
(7 – 8)	518	24	172	1.85 (0.96,3.57)	2.02 (0.94,4.32)	0.0660
(9 – 11)	631	43	244	2.70 (1.48,4.93)	1.89 (0.94,3.80)	0.0699
(12 – 16)	215	26	71	5.12 (2.64,9.93)	4.11 (1.85,9.14)	0.0004
Rectal Cancer						
				CIMP+		

Score[1]	N			OR (95%CI)[2]	OR (95% CI)[2]	Heterogeneity
	Controls	Case+	Cases−	Case+ vs Controls CIMP+	CASE+ vs. CASE−	p value[3]
(0 – 3)	345	6	152	1.00	1.00	
(4 – 5)	327	19	163	3.46 (1.36,8.79)	2.95 (1.13,7.69)	0.0185
(6 – 7)	219	23	114	6.30 (2.51,15.80)	5.00 (1.93,12.95)	0.0003
(8 – 11)	68	11	43	9.71 (3.45,27.32)	11.31 (3.05,41.95)	<.0001
				TP53		
(0 – 0)	226	55	75	1.00	1.00	
(1 – 2)	315	63	77	0.77 (0.52,1.16)	1.13 (0.67,1.89)	0.6562
(3 – 4)	266	79	72	1.18 (0.80,1.74)	1.70 (1.03,2.82)	0.0376
(5 – 6)	113	55	39	1.93 (1.24,2.99)	2.08 (1.15,3.76)	0.0145
(7 – 12)	39	25	18	2.58 (1.44,4.63)	1.73 (0.81,3.70)	0.1562
				CIMP+& *KRAS*		
(0 – 5)	663	5	234	1.00	1.00	
(6 – 8)	227	8	70	4.72 (1.53,14.59)	5.20 (1.59,16.97)	0.0057
(9 – 14)	69	8	33	15.81 (5.01,49.89)	12.93 (3.80,43.97)	<.0001

[1] SNPs included in summary score

Colon CIMP+: *BMP4* (rs17563 and (rs762642), *BMPR1A* (rs12765929), *BMPR2* (rs12477602 and rs4303700);

Rectal CIMP+: *BMP1* (rs4076873), *BMP2* (rs235770), *BMPR1B* (rs11947569, rs3821968 and rs7662504), *GDF10* (rs762454);

Colon *KRAS: BMPR1B* (rs17616243, rs2719176, rs4490463, rs7698964, rs9307147), *GDF10* (rs2853838);

Rectal *TP53: BMP2* (rs1979855, rs235770), *BMPR1B* (rs11947569), *BMPR2* (rs17199235, rs2228545, rs4675278);

Colon MSI: BMP1 (rs3857979, rs4075478), BMP4 (rs17563), BMPR1A (rs6586034), *BMPR1B* (rs12508087, rs4490463, rs6849425), *BMPR2* (rs12621870, rs4675278);

Colon CIMP+ & *KRAS: BMP1* (rs3924231), *BMPR2* (rs13430786), *GDF10* (rs7093975;

Rectal CIMP+ & *KRAS: BMP1* (rs4076873), *BMP2* (rs235770), *BMPR1B* (rs1863652, rs2214395, rs3821968), *BMPR2* (rs1199496), *GDF10* (rs1902724);

Colon CIMP+ & MSI: *BMP1* (rs13257482, rs3857979), *BMP2* (rs1005464, rs3178250), *BMP4* (rs17563), *BMPR1B* (rs12508087, rs4490463), *BMPR2* (rs4675278);.

[2] Odds Ratios (OR) and 95% Confidence Intervals (CI) estimated from multiple logistic regression models adjusting for age, center, ethnicity/race, and sex

[3] p for heterogeneity based on regression models comparing cases with and without tumor molecular phenotype