Original Contribution

Variation in the Association Between Colorectal Cancer Susceptibility Loci and Colorectal Polyps by Polyp Type

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We conducted a case-control study of the association between subsets of colorectal polyps, including adenomas and serrated polyps, and single-nucleotide polymorphisms (SNPs) related to colorectal cancer through prior genome-wide association studies (GWAS). Participants were enrollees in the Group Health Cooperative (Seattle, Washington) aged 24–79 years who received a colonoscopy from 1998 to 2007, donated a buccal or blood sample, and completed a structured questionnaire. We performed genotyping of 13 colorectal cancer susceptibility SNPs. Polytomous logistic regression models were used to estimate odds ratios and 95% confidence intervals for associations between polyps and the colorectal cancer risk allele for each SNP under a log-additive model. Analyses included 781 controls, 489 cases with adenoma, 401 cases with serrated polyps, and 188 cases with both polyp types. The following SNPs were associated with advanced adenomas: rs10936599, rs10795668, rs16892766, and rs9929218 ($P < 0.05$). For nonadvanced adenomas and for serrated polyps overall, only rs961253 was statistically significant ($P < 0.05$). These associations were in the same directions as those in prior colorectal cancer GWAS. No SNP was significantly associated with hyperplastic polyps, and only rs6983267 was significantly associated with sessile serrated polyps, but this association was opposite of that found in colorectal cancer GWAS. Our results suggest that the association between colorectal cancer susceptibility SNPs and colorectal polyps varies by polyp type.

adenoma; colorectal cancer; colorectal polyps; genome-wide association studies; serrated polyps; single-nucleotide polymorphisms

Abbreviations: *BMP2*, bone morphogenetic protein 2 gene; *BRAF*, v-Raf murine sarcoma viral oncogene homolog B gene; *CDH1*, cadherin 1 gene; CI, confidence interval; *DIP2B*, disco-interacting protein 2 homolog B (*Drosophila*) gene; *DUSP10*, dual specificity phosphatase 10 gene; *EIF3H*, eukaryotic translation initiation factor 3, subunit H, gene; GWAS, genome-wide association studies; *MYC*, v-Myc avian myelocytomatosis viral oncogene homolog gene; *MYNN*, myoneurin gene; OR, odds ratio; *RHPN2*, rhophilin, Rho GTPase binding protein 2 gene; rs, reference SNP; SNP, single-nucleotide polymorphism; SSPs, sessile serrated polyps.

Colorectal cancer is a heterogeneous disease, and the majority of colorectal cancers develop via polyp precursors (1). Just as there is heterogeneity among colorectal adenocarcinomas, there is also diversity in colorectal polyps (2). This heterogeneity in colorectal cancers and polyps is partially the result of different risk factors, including both environmental and genetic risk factors, which drive colorectal neoplastic progression along distinct pathways (1).

The adenoma-carcinoma pathway is the most common colorectal cancer pathway, accounting for approximately 75% of colorectal cancers (3). Collectively called adenomas or conventional adenomas, polyps in this pathway include tubular adenomas, tubulovillous adenomas, and villous adenomas. Although most adenomas will not progress to cancer (4), some adenomas grow large, become increasingly dysplastic, and eventually develop into malignant adenocarcinomas (5).

Adenomas that are ≥10 mm in diameter or have high-grade dysplasia or villous components are considered "advanced" and have the greatest risk of developing into a malignancy (6).

A separate pathway to colorectal cancer, the "serrated pathway," involves serrated polyps as important precursor lesions and gives rise to approximately 12%–15% of colorectal cancers (7–9). Serrated polyps have a saw-toothed appearance and include hyperplastic polyps, sessile serrated polyps (SSPs) (also known as sessile serrated adenomas), and traditional serrated adenomas (9). Most hyperplastic polyps are considered low-risk lesions that will not develop into cancer. However, both SSPs and traditional serrated adenomas are associated with an increased risk of colorectal cancer and are considered high-risk, or advanced, serrated polyps (2, 9).

Several studies have found differential associations between different colorectal polyp subtypes and colorectal cancer risk factors, such as smoking and obesity (10–16). There are also prior studies that were aimed at determining genetic factors differentially associated with specific polyp types (10, 17–20). These types of studies can provide insight into the different genetic mechanisms that are important for initiation and promotion in distinct colorectal cancer pathways. They may also help elucidate the function of specific loci that have been identified as colorectal cancer susceptibility loci through prior genome-wide association studies (GWAS) (21, 22). The objective of this study was to determine the association between previously reported GWAS-identified colorectal cancer susceptibility loci and polyp subtypes, including adenomas, serrated polyps, and advanced and nonadvanced polyps.

METHODS

Study population

Details on this study population were previously reported (11). Participants were enrollees in an integrated health-care delivery system in western Washington State (Group Health Cooperative, Seattle, Washington) aged 24–79 years who underwent an index colonoscopy for any indication between 1998 and 2007 and donated a buccal-cell or blood sample for genotyping analysis. Study recruitment took place in 2 phases, with phase 1 occurring in 1998–2003 and phase 2 occurring in 2004–2007. Persons who had undergone a colonoscopy less than 1 year prior to the index colonoscopy, persons with inadequate bowel preparation for the index colonoscopy, and persons with a prior or new diagnosis of colorectal cancer, a familial colorectal cancer syndrome (such as familial adenomatous polyposis), or another colorectal disease were ineligible. Patients diagnosed with adenomas or serrated polyps and persons who were polyp-free at the index colonoscopy (controls) were systematically recruited during both phases of recruitment. Approximately 75% agreed to participate and provided written informed consent. Based on medical records, persons who agreed to participate and those who refused study participation were similar with respect to age, sex, and colorectal polyp status. Study protocols were approved by the institutional review boards of the Group Health Cooperative and the Fred Hutchinson Cancer Research Center (Seattle, Washington).

Study questionnaire and medical record abstraction

Participants completed a structured questionnaire that elicited information on their demographic characteristics, including race/ethnicity, and personal risk factors for colorectal cancer (11). Participant sex and age at the index colonoscopy were confirmed through standardized medical record abstraction. The size of the index polyp was determined through abstraction of endoscopy and pathology reports.

Standardized pathology review

Two study pathologists worked in tandem to conduct a standardized pathology review of clinical biopsy specimens, which had previously been fixed in paraffin, cut and mounted onto slides, and stained with hematoxylin and eosin. Using established protocols and criteria, these pathologists classified polyps as belonging to one of 6 types: 1) tubular adenomas; 2) tubulovillous adenomas (having ≥20% villous components); 3) hyperplastic polyps; 4) SSPs; 5) traditional serrated adenomas; and 6) other colorectal polyps. Disagreements between study pathologists were reconciled through re-review by both pathologists and by referral to a standard training set of polyp slides.

Case-control classification

Four groups of study participants were defined on the basis of the clinical findings at endoscopy and the standardized pathology review. If a participant had at least 1 index tubular or tubulovillous adenoma and no serrated polyps, he/she was classified as an adenoma case. Participants with hyperplastic polyps, traditional serrated adenomas, or SSPs and no synchronous adenomas were classified as serrated polyp cases (23). Cases with both adenomas and serrated polyps were placed into a separate category. Controls were persons who had no colorectal pathology identified and no biopsies collected during the index colonoscopy.

Classification of lesion severity

Adenomas were classified as advanced if they 1) were ≥10 mm in diameter according to the endoscopic determination of polyp size or 2) had ≥20% villous components or high-grade dysplasia according to the standard pathology review. Among serrated polyps, SSPs were considered advanced lesions and hyperplastic polyps were considered nonadvanced lesions. Notably, traditional serrated adenomas are also a distinct type of advanced serrated polyp. Because traditional serrated adenomas and SSPs tend to exhibit different molecular markers and have differing distributions in the colon and rectum, these polyps are generally hypothesized to have distinct developmental trajectories within the serrated pathway (9). Ideally, we would categorize traditional serrated adenomas into their own advanced serrated case group; however, there were only 14 cases with traditional serrated adenomas, so these were excluded from analyses of lesion severity.

Selection of single-nucleotide polymorphisms and genotyping

We selected 13 single-nucleotide polymorphisms (SNPs) that were associated with colorectal cancer in prior GWAS at a significance level of $P < 5 \times 10^{-8}$ and for which the association was replicated in a separate study population (24–29). Table 1 lists these SNPs from prior GWAS and the particulars of their associations with colorectal cancer. We extracted genomic DNA from lymphocytes during phase 1 of data collection and from buccal samples during phase 2, using the Qiagen QIAamp DNA extraction kit (Qiagen Inc., Valencia, California). Extracted DNA was stored as pellets at −80°C until the pellets were genotyped. We used the Illumina GoldenGate assay with VeraCode microbeads and the BeadXpress reader (Illumina, Inc., San Diego, California) to perform multiplex genotyping of SNPs (30). For quality control and to monitor for possible contamination between samples, we included wells with reagents only and genotyped a 3% random sample of study participants in duplicate. Genotyping results were similar between duplicate samples, and all SNPs were in Hardy-Weinberg equilibrium among controls ($P < 0.05$ for each SNP).

Statistical analyses

For each SNP, we defined the risk allele as the allele associated with an increased risk of colorectal cancer in prior GWAS. Genotypes were then coded as 0, 1, or 2, according to the number of risk alleles that were present for each SNP. This coding was done to make it easy to see whether associations were in the same direction as prior colorectal cancer GWAS or in the opposite direction. We also constructed a risk-allele score for each individual which was the sum of risk alleles present in that individual across the 13 SNPs of interest (possible values ranged from 0 to 26). Genotypes were analyzed with log-additive models, and the risk-allele score was analyzed as a continuous variable. We used polytomous logistic regression models to compare each polyp case group with the polyp-free control group and to estimate adjusted odds ratios and 95% confidence intervals for the associations between polyp subtypes and increasing numbers of risk alleles for each SNP and for the risk-allele score (31). These same models were used to compare case groups with one another, and we evaluated the Wald P value for the comparison of heterogeneity between case groups for each SNP and the risk-allele score. Results of all regression analyses were adjusted for study phase, age, sex, and race/ethnicity. Among eligible participants, data were complete for these variables. All statistical analyses were performed using Stata 12.0 (StataCorp LP, College Station, Texas).

RESULTS

Of the 2,506 eligible study participants with complete questionnaire and pathology data, 1,904 gave a buccal or

Table 1. Colorectal Cancer Susceptibility Loci Identified in Previously Published Genome-Wide Association Studies

Gene	Position	rs No.	Risk Allele	Risk Allele Frequency	Odds Ratio	P Value	First Author, Year (Reference No.)
DUSP10	1q41	rs6691170	T	0.36	1.06	1×10^{-9}	Houlston, 2010 (27)
MYNN	3q26.2	rs10936599	C	0.75	1.04	3×10^{-8}	Houlston, 2010 (27)
EIF3H	8q23.3	rs16892766	C	0.07	1.27	3×10^{-18}	Tomlinson, 2008 (25)
MYC	8q24	rs6983267	G	0.49	1.27	1×10^{-14}	Tomlinson, 2007 (24)
	10p14[a]	rs10795668	G	0.67	1.12	3×10^{-13}	Tomlinson, 2008 (25)
	11q23.1[a]	rs3802842	C	0.30	1.11	6×10^{-10}	Tenesa, 2008 (28)
DIP2B	12q13.13	rs11169552	C	0.73	1.09	2×10^{-10}	Houlston, 2010 (27)
BMP4	14q22.2	rs4444235	C	0.46	1.11	8×10^{-10}	Houlston, 2008 (26)
CRAC1/GREM1	15q13.3	rs4779584	T	0.19	1.18	2×10^{-8}	Peters, 2012 (29)
CDH1	16q22.1	rs9929218	G	0.71	1.10	1×10^{-8}	Houlston, 2008 (26)
SMAD7	18q21.1	rs4939827	T	0.52	1.20	8×10^{-28}	Tenesa, 2008 (28)
RHPN2	19q13.1	rs10411210	C	0.90	1.15	5×10^{-9}	Houlston, 2008 (26)
BMP2	20p12.3	rs961253	A	0.36	1.12	2×10^{-10}	Houlston, 2008 (26)

Abbreviations: BMP, bone morphogenetic protein; *BMP2*, bone morphogenetic protein 2 gene; *BMP4*, bone morphogenetic protein 4 gene; *CDH1*, cadherin 1 gene; *CRAC1*, colorectal adenoma and carcinoma 1 gene; DAN, deadenylating nuclease; *DIP2B*, disco-interacting protein 2 homolog B (*Drosophila*) gene; *DUSP10*, dual specificity phosphatase 10 gene; *EIF3H*, eukaryotic translation initiation factor 3, subunit H, gene; *GREM1*, gremlin 1, DAN family BMP antagonist gene; GTPase, guanosine triphosphatase; *MYC*, v-Myc avian myelocytomatosis viral oncogene homolog gene; *MYNN*, myoneurin gene; *RHPN2*, rhophilin, Rho GTPase binding protein 2 gene; rs, reference SNP; *SMAD7*, SMAD[b] family member 7 gene; SNP, single-nucleotide polymorphism.

[a] No known gene association with this locus.

[b] The SMAD proteins are homologs of both the *Drosophila* protein "mothers against decapentaplegic" (MAD) and the *Caenorhabditis elegans* protein SMA (from the gene *sma*, for small body size). The abbreviation is a combination of the two (Human Genome Organization Gene Nomenclature Committee (http://www.genenames.org/)).

blood sample and had genomic DNA available for genotyping analyses. The success rate for genotyping was approximately 98%. This resulted in 1,859 study participants (340 from phase 1 and 1,519 from phase 2) with genotyping data available, comprising 489 cases with adenomas only, 401 cases with serrated polyps only, 188 cases with synchronous adenomas and serrated polyps, and 781 polyp-free controls (Table 2). Compared with controls, cases with adenoma were more likely to be male and to be older. Serrated polyp cases were similar to controls with regard to age, sex, and race/ethnicity.

Colorectal cancer susceptibility SNPs in adenomas versus serrated polyps

Of the 13 colorectal cancer susceptibility loci analyzed, only those on the cadherin 1 gene (*CDH1*/16q22.1 [gene symbol/chromosomal position]; rs9929218 [reference SNP]) and the bone morphogenetic protein 2 gene (*BMP2*/20p12.3; rs961253) were statistically significantly associated with adenomas (Table 3). Comparing adenoma cases with controls, the colorectal cancer risk allele (G) for *CDH1*/16q22.1 (rs9929218) was associated with increased odds of adenoma (odds ratio (OR) = 1.24, 95% confidence interval (CI): 1.04, 1.50), as was the risk allele (A) for *BMP2*/20p12.3 (rs961253) (OR = 1.23, 95% CI: 1.03, 1.46). In addition, adenomas were positively associated with the risk-allele score (per risk-allele increase, OR = 1.07, 95% CI: 1.01, 1.13). The only SNP that was statistically significantly associated with serrated polyps was *BMP2*/20p12.3 (rs961253) (OR = 1.24, 95% CI: 1.03, 1.50), and there was no association between the risk-allele score and serrated polyps (per risk-allele increase, OR = 1.00, 95% CI: 0.94, 1.06). No SNPs were statistically significantly associated with synchronous adenomas or serrated polyps (at $\alpha = 0.05$). Only the associations for 11q23.1 (rs3802842), *CDH1*/16q22.1 (rs9929218), and the risk-allele score were statistically significantly different between adenomas and serrated polyps ($P \le 0.05$ for each).

Colorectal cancer susceptibility SNPs in advanced vs. nonadvanced adenomas

Of the 677 cases with at least 1 adenoma, 181 had 1 or more advanced adenomas and 296 had nonadvanced adenomas only; 200 were excluded because of missing data on polyp size (Table 4). When comparing nonadvanced adenoma cases with controls, only the colorectal cancer risk allele (A) for *BMP2*/20p12.3 (rs961253) was statistically significantly associated with nonadvanced adenomas (OR = 1.27, 95% CI: 1.04, 1.55). When comparing advanced adenomas with controls, the risk allele (C) for the myoneurin gene (*MYNN*/3q26.2; rs10936599) (OR = 1.32, 95% CI: 1.01, 1.74), the risk allele (C) for the eukaryotic translation initiation factor 3, subunit H, gene (*EIF3H*/8q23.3; rs16892766) (OR = 1.48, 95% CI: 1.02, 2.16), the risk allele (G) for 10p14 (rs10795668) (OR = 1.50, 95% CI: 1.15, 1.96), and the risk allele (G) for *CDH1*/16q22.1 (rs9929218) (OR = 1.44, 95% CI: 1.09, 1.89) were statistically significantly associated with advanced adenomas. Of the SNPs evaluated, only the risk allele (G) for 10p14 (rs10795668) was differentially associated with advanced adenomas in comparison with nonadvanced adenomas ($P = 0.01$). In addition, the association between the risk-allele score and adenoma varied significantly by lesion severity ($P = 0.05$): Per risk-allele increase, the odds ratio was 1.04 (95% CI: 0.98, 1.10) for nonadvanced adenomas and 1.13 (95% CI: 1.05, 1.22) for advanced adenomas.

Colorectal cancer susceptibility SNPs in SSPs versus hyperplastic polyps

Of the 589 cases with at least 1 serrated polyp, 137 had 1 or more SSPs, 438 had hyperplastic polyps but no SSPs, and 14

Table 2. Demographic Characteristics of Participants in a Study of Colorectal Cancer Susceptibility Loci and Colorectal Polyps, by Case-Control Status, Group Health Cooperative, Seattle, Washington, 1998–2007

| | | | Cases With Colorectal Polyps | | | | | |
| | Controls (*n* = 781) | | Adenomas Only (*n* = 489) | | Serrated Polyps Only (*n* = 401) | | Adenomas + Serrated Polyps (*n* = 188) | |
	No.	%	No.	%	No.	%	No.	%
Age group, years								
24–49	79	10	29	6	20	5	8	4
50–60	331	42	210	43	204	51	72	38
61–70	265	34	173	35	131	33	82	44
71–79	106	14	77	16	46	11	26	14
Male sex	312	40	256	52	172	43	106	56
Race/ethnicity								
Caucasian	679	87	418	85	357	89	162	86
African-American	22	3	12	3	3	1	4	2
Asian-American	34	4	26	5	16	4	8	4
Other	46	6	33	7	25	6	14	7

Table 3. Odds of Colorectal Polyps Among Persons With GWAS-Identified Colorectal Cancer Susceptibility Loci in Polytomous Logistic Regression Analysis, by Polyp Subtype, Group Health Cooperative, Seattle, Washington, 1998–2007

| | | | | | Cases With Colorectal Polyps | | | | | | | | | P for Comparison Between Case Groups | | |
| Gene[a] | Position | rs No. | Risk Allele[b] | RAF in Controls[c] (n = 781) | Adenomas Only (n = 489) | | | Serrated Polyps Only (n = 401) | | | Adenomas + Serrated Polyps (n = 188) | | | | | |
					RAF	OR[d]	95% CI	RAF	OR[d]	95% CI	RAF	OR[d]	95% CI	ADs vs. SPs	ADs vs. ADs + SPs	SPs vs. ADs + SPs
DUSP10	1q41	rs6691170	T	0.36	0.33	0.89	0.75, 1.05	0.34	0.89	0.74, 1.08	0.34	0.92	0.73, 1.18	0.96	0.76	0.80
MYNN	3q26.2	rs10936599	C	0.73	0.76	1.18	0.98, 1.42	0.74	0.99	0.81, 1.20	0.78	1.27	0.98, 1.67	0.11	0.57	0.08
EIF3H	8q23.3	rs16892766	C	0.08	0.08	1.01	0.75, 1.36	0.08	1.01	0.73, 1.40	0.09	1.14	0.77, 1.70	0.99	0.58	0.59
MYC	8q24	rs6983267	G	0.53	0.50	0.93	0.79, 1.09	0.49	0.91	0.76, 1.09	0.53	1.03	0.82, 1.29	0.85	0.39	0.33
	10p14[e]	rs10795668	G	0.68	0.70	1.14	0.96, 1.36	0.69	1.09	0.90, 1.33	0.69	1.06	0.83, 1.36	0.69	0.59	0.84
	11q23.1[e]	rs3802842	C	0.29	0.31	1.08	0.91, 1.28	0.26	0.86	0.71, 1.05	0.28	0.95	0.74, 1.21	0.03	0.32	0.50
DIP2B	12q13.13	rs11169552	C	0.71	0.73	1.12	0.94, 1.34	0.73	1.10	0.90, 1.34	0.73	1.11	0.86, 1.42	0.85	0.93	0.95
BMP4	14q22.2	rs4444235	C	0.49	0.45	0.87	0.74, 1.02	0.49	1.03	0.86, 1.23	0.47	0.94	0.75, 1.18	0.09	0.52	0.48
CRAC1/GREM1	15q13.3	rs4779584	T	0.23	0.24	1.06	0.88, 1.28	0.22	1.01	0.82, 1.39	0.24	1.07	0.82, 1.38	0.67	0.99	0.72
CDH1	16q22.1	rs9929218	G	0.71	0.75	1.24	1.04, 1.50	0.70	1.00	0.82, 1.21	0.74	1.23	0.95, 1.60	0.05	0.94	0.15
SMAD7	18q21.1	rs4939827	T	0.50	0.52	1.09	0.93, 1.29	0.49	0.93	0.76, 1.11	0.52	1.09	0.86, 1.37	0.10	0.97	0.22
RHPN2	19q13.1	rs10411210	C	0.89	0.90	1.14	0.87, 1.49	0.89	1.02	0.77, 1.36	0.89	1.01	0.70, 1.45	0.49	0.53	0.95
BMP2	20p12.3	rs961253	A	0.32	0.37	1.23	1.03, 1.46	0.38	1.24	1.03, 1.50	0.36	1.16	0.92, 1.48	0.90	0.67	0.61
Risk-allele score[f]			NA	NA	NA	1.07	1.01, 1.13	NA	1.00	0.94, 1.06	NA	1.07	0.99, 1.15	0.03	0.97	0.09

Abbreviations: AD, adenoma; CI, confidence interval; GWAS, genome-wide association studies; NA, not applicable; OR, odds ratio; RAF, risk allele frequency; rs, reference SNP; SNP, single-nucleotide polymorphism; SP, serrated polyp.

[a] For gene abbreviations, see Table 1.
[b] Allele associated with an increased risk of colorectal cancer in prior GWAS.
[c] Reference category (OR = 1).
[d] Odds per risk-allele increase; adjusted for study phase, age, race/ethnicity, and sex.
[e] No known gene association with this locus.
[f] Combined number of risk alleles across all SNPs in Table 3; possible values range from 0 to 26.

Table 4. Odds of Nonadvanced and Advanced Adenomas Associated With GWAS-Identified Colorectal Cancer Susceptibility Loci in Polytomous Logistic Regression Analysis, Group Health Cooperative, Seattle, Washington, 1998–2007[a]

Gene[b]	Position	rs No.	Risk Allele[c]	RAF in Controls[d] (n = 781)	Cases With Colorectal Polyps						P for Advanced Adenomas vs. Nonadvanced Adenomas
					Nonadvanced Adenomas (n = 296)			Advanced Adenomas (n = 181)			
					RAF	OR[e]	95% CI	RAF	OR[e]	95% CI	
DUSP10	1q41	rs6691170	T	0.36	0.33	0.91	0.74, 1.10	0.33	0.84	0.65, 1.07	0.59
MYNN	3q26.2	rs10936599	C	0.73	0.75	1.10	0.88, 1.36	0.78	1.32	1.01, 1.74	0.22
EIF3H	8q23.3	rs16892766	C	0.08	0.08	1.05	0.74, 1.48	0.12	1.48	1.02, 2.16	0.12
MYC	8q24	rs6983267	G	0.53	0.50	0.93	0.77, 1.12	0.53	1.04	0.83, 1.31	0.39
	10p14[f]	rs10795668	G	0.68	0.68	1.03	0.84, 1.26	0.76	1.50	1.15, 1.96	0.01
	11q23.1[f]	rs3802842	C	0.29	0.31	1.08	0.88, 1.32	0.31	1.12	0.88, 1.43	0.79
DIP2B	12q13.13	rs11169552	C	0.71	0.72	1.08	0.87, 1.34	0.74	1.19	0.91, 1.55	0.53
BMP4	14q22.2	rs4444235	C	0.49	0.46	0.90	0.74, 1.09	0.45	0.88	0.70, 1.11	0.88
CRAC1/ GREM1	15q13.3	rs4779584	T	0.23	0.24	1.07	0.86, 1.33	0.26	1.22	0.93, 1.59	0.39
CDH1	16q22.1	rs9929218	G	0.71	0.73	1.13	0.91, 1.40	0.77	1.44	1.09, 1.89	0.13
SMAD7	18q21.1	rs4939827	T	0.50	0.51	1.04	0.86, 1.26	0.49	0.99	0.78, 1.25	0.70
RHPN2	19q13.1	rs10411210	C	0.89	0.90	1.10	0.81, 1.50	0.87	0.88	0.62, 1.25	0.28
BMP2	20p12.3	rs961253	A	0.32	0.37	1.27	1.04, 1.55	0.36	1.18	0.92, 1.51	0.60
Risk-allele score[g]		NA	NA	NA	NA	1.04	0.98, 1.10	NA	1.13	1.05, 1.22	0.05

Abbreviations: CI, confidence interval; GWAS, genome-wide association studies; NA, not applicable; OR, odds ratio; RAF, risk allele frequency; rs, reference SNP; SNP, single-nucleotide polymorphism.

[a] Analysis excludes 200 tubular adenoma cases that were missing data on polyp size.

[b] For gene abbreviations, see Table 1.

[c] Allele associated with an increased risk of colorectal cancer in prior GWAS.

[d] Reference category (OR = 1).

[e] Odds per risk-allele increase; adjusted for study phase, age, race/ethnicity, and sex.

[f] No known gene association with this locus.

[g] Combined number of risk alleles across all SNPs in Table 4; possible values range from 0 to 26.

were excluded because they had traditional serrated adenomas (Table 5). None of the 13 SNPs evaluated were statistically significantly associated with hyperplastic polyps. Only the risk allele (G) for the v-Myc avian myelocytomatosis viral oncogene homolog gene (MYC/8q24; rs6983267) was associated with SSPs (OR = 0.64, 95% CI: 0.49, 0.83), and the association between this SNP and SSPs was statistically significantly different from the association between MYC/8q24 (rs6983267) and hyperplastic polyps (P < 0.01). Although the risk-allele score was not associated with hyperplastic polyps (per risk-allele increase, OR = 1.06, 95% CI: 0.99, 1.12) or with SSPs (per risk-allele increase, OR = 0.95, 95% CI: 0.87, 1.03), there was evidence for heterogeneity in the association between risk-allele score and serrated polyps by lesion severity (P = 0.01).

DISCUSSION

Using the combined risk-allele score to measure the overall association between the GWAS-identified SNPs evaluated in this study and colorectal polyps, our results suggest that, overall, these SNPs are involved in early carcinogenesis for the adenoma-carcinoma pathway but not for the serrated pathway. In addition, the association between the combined risk-allele score and colorectal adenomas was stronger for advanced adenomas than for nonadvanced adenomas, indicating that these SNPs may play a larger role in promotion of colorectal cancer than in its initiation. In addition to using the risk-allele score to determine the overall association between these colorectal cancer susceptibility SNPS and each polyp type, we were able to evaluate associations of colorectal polyps with each individual locus.

Colorectal cancer susceptibility SNPs and adenomas

We found that the following 5 SNPs were statistically significantly associated (P < 0.05) with adenomas or advanced adenomas: MYNN/3q26.2 (rs10936599), 10p14 (rs10795668), EIF3H/8q23.3 (rs16892766), CDH1/16q22.1 (rs9929218), and BMP2/20p12.3 (rs961253). All of these associations were in the same direction as had been previously reported for colorectal cancer (24–29). Several prior studies using adenoma cases and polyp-free controls have reported on the association between adenomas and select GWAS-identified colorectal cancer susceptibility SNPs (20, 21, 24, 32–34), and 3 of these studies used a panel of SNPs that included the SNPs evaluated in our analyses (20, 21, 34). Although these studies were conducted in separate study populations and

Table 5.   Odds of Hyperplastic Polyps and Sessile Serrated Polyps Associated With GWAS-Identified Colorectal Cancer Susceptibility Loci in Polytomous Logistic Regression Analysis, Group Health Cooperative, Seattle, Washington, 1998–2007[a]

Gene[b]	Position	rs No.	Risk Allele[c]	RAF in Controls[d] (n = 781)	Cases With Colorectal Polyps						P for SSPs vs. HPs
					Hyperplastic Polyps (n = 438)			Sessile Serrated Polyps (n = 137)			
					RAF	OR[e]	95% CI	RAF	OR[e]	95% CI	
DUSP10	1q41	rs6691170	T	0.36	0.34	0.95	0.79, 1.13	0.32	0.82	0.62, 1.08	0.31
MYNN	3q26.2	rs10936599	C	0.73	0.76	1.14	0.94, 1.39	0.71	0.92	0.70, 1.23	0.16
EIF3H	8q23.3	rs16892766	C	0.08	0.08	1.07	0.78, 1.46	0.07	0.91	0.55, 1.50	0.53
MYC	8q24	rs6983267	G	0.53	0.53	1.03	0.87, 1.22	0.41	0.64	0.49, 0.83	<0.01
	10p14[f]	rs10795668	G	0.68	0.70	1.13	0.94, 1.37	0.67	1.00	0.76, 1.32	0.40
	11q23.1[f]	rs3802842	C	0.29	0.26	0.88	0.73, 1.07	0.27	0.95	0.71, 1.26	0.61
DIP2B	12q13.13	rs11169552	C	0.71	0.72	1.07	0.88, 1.29	0.74	1.19	0.89, 1.59	0.51
BMP4	14q22.2	rs4444235	C	0.49	0.49	1.02	0.85, 1.21	0.50	1.07	0.82, 1.39	0.73
CRAC1/GREM1	15q13.3	rs4779584	T	0.23	0.23	1.06	0.86, 1.30	0.23	1.05	0.77, 1.43	0.99
CDH1	16q22.1	rs9929218	G	0.71	0.73	1.13	0.93, 1.37	0.69	0.92	0.69, 1.21	0.17
SMAD7	18q21.1	rs4939827	T	0.50	0.51	1.05	0.88, 1.26	0.46	0.85	0.65, 1.10	0.12
RHPN2	19q13.1	rs10411210	C	0.89	0.89	1.00	0.76, 1.33	0.91	1.19	0.77, 1.85	0.47
BMP2	20p12.3	rs961253	A	0.32	0.37	1.19	0.99, 1.43	0.37	1.19	0.90, 1.56	0.99
Risk-allele score[g]		NA	NA	NA	NA	1.06	0.99, 1.12	NA	0.95	0.87, 1.03	0.01

Abbreviations: CI, confidence interval; GWAS, genome-wide association studies; HP, hyperplastic polyp; NA, not applicable; OR, odds ratio; RAF, risk allele frequency; rs, reference SNP; SNP, single-nucleotide polymorphism; SSP, sessile serrated polyp.

[a] Analysis excludes 14 traditional serrated adenoma cases.
[b] For gene abbreviations, see Table 1.
[c] Allele associated with an increased risk of colorectal cancer in prior GWAS.
[d] Reference category (OR = 1).
[e] Odds per risk-allele increase; adjusted for study phase, age, race/ethnicity, and sex.
[f] No known gene association with this locus.
[g] Combined number of risk alleles across all SNPs in Table 5; possible values range from 0 to 26.

used different protocols for ascertainment of case-control status, each study found associations between adenomas and 3 or more of the SNPs evaluated in the present study. In addition, all 5 of the SNPs we found to be statistically significantly associated with adenomas or advanced adenomas were associated with adenomas at $P < 0.05$ in at least 1 of these prior studies. Of the 13 SNPs we evaluated, only 3 were consistently null across all studies—namely those on the dual specificity phosphatase 10 gene (*DUSP10*/1q41; rs6691170), the disco-interacting protein 2 homolog B (*Drosophila*) gene (*DIP2B*/12q13.13; rs11169552), and the rhophilin, Rho GTPase binding protein 2 gene (*RHPN2*/19q13.1; rs10411210). Thus, in combination with the results from prior studies, our findings support the thesis that many of the GWAS-identified colorectal cancer susceptibility loci are associated with early events in colorectal carcinogenesis for the adenoma-carcinoma pathway.

In the adenoma-carcinoma pathway, a lesion progresses to cancer in a series of steps, from a small, nonadvanced adenoma to an advanced adenoma and eventually an invasive carcinoma (35). Factors that promote carcinogenesis, such as increasing age, are positively associated with advanced adenoma prevalence (36), and factors that interfere with promotion, such as a prior history of colonoscopy, are negatively associated with advanced adenomas (37). Thus, by evaluating

whether the association between adenomas and GWAS-identified colorectal cancer SNPs varies according to lesion severity, we aimed to gain insight into whether these SNPs were more important for cancer initiation or cancer promotion. Only 1 other study has reported on the association between the GWAS-identified colorectal cancer SNPs analyzed in the present study and both advanced adenomas and nonadvanced adenomas (20). Similar to our results, those authors reported that advanced adenomas tended to have a stronger association with GWAS-identified colorectal cancer susceptibility SNPs than nonadvanced adenomas (20). Also consistent with our results, the authors found that the association between adenomas and a composite colorectal cancer susceptibility risk-allele score varied according to lesion severity. This suggests that the SNPs evaluated in this study tend to be important in colorectal cancer promotion.

Colorectal cancer susceptibility SNPs and serrated polyps

The only SNP that was associated with serrated polyps, overall, was *BMP2*/20p12.3 (rs961253). This SNP was similarly associated with adenomas, and although the specific function of the rs961253 SNP is unknown, it is in close

proximity to the *BMP2* gene, which regulates the expression of bone morphogenetic protein 2 (38). Bone morphogenetic protein 2 is part of the transforming growth factor β superfamily of proteins and is involved in the regulation of apoptosis in colonic epithelial cells (39). Thus, the rs961253 SNP may play a role in both the adenoma-carcinoma and serrated pathways to colorectal cancer through changes in the rate or function of colonic epithelial apoptosis.

To our knowledge, this is the first study to have evaluated the association between GWAS-identified colorectal cancer susceptibility SNPs and SSPs. SSPs are considered advanced precursor lesions in the serrated pathway to colorectal cancer (9). In contrast to our results for advanced adenomas, we found no association between the composite risk-allele score and SSPs. However, we observed a statistically significant inverse association between the risk allele (G) for *MYC*/8q24 (rs6983267) and SSPs that was in the direction opposite that seen for this locus and colorectal cancer (24). This was an unexpected finding, because this SNP has been associated with several cancers, including colorectal, ovarian, and prostate cancer, and all of these cancers are positively associated with the risk allele (G) (40). Thus, our result suggesting an inverse association between SSPs and the risk allele for *MYC*/8q24 (rs6983267) may be a chance finding and needs to be replicated in future studies.

Only 1 other study has examined these SNPs in relation to the most common type of serrated polyp, hyperplastic polyps. Zhang et al. (20) found that 3 SNPs were associated with hyperplastic polyps: *MYNN*/3q26.2 (rs10936599), colorectal adenoma and carcinoma 1 (*CRAC1*)/gremlin 1 (*GREM1*)/15q13.3 (rs4779584), and *RHPN2*/19q13.1 (rs10411210). In contrast to Zhang et al., we observed no statistically significant associations between colorectal cancer susceptibility SNPs and hyperplastic polyps. We also found no association between the composite risk-allele score and serrated polyps overall, hyperplastic polyps, or SSPs. This is again in contrast to the findings of Zhang et al., who reported a positive association between a composite risk-allele score and hyperplastic polyps ($P < 0.01$) (20). However, as we did, Zhang et al. found that the association between hyperplastic polyps and the colorectal cancer susceptibility SNPs tended to be weaker than the association between these SNPs and adenomas.

The null association we observed between each type of serrated polyp and the composite risk-allele score was not surprising in light of the fact that approximately 75% of colorectal cancers develop along the adenoma-carcinoma pathway (3). To date, colorectal cancer GWAS have either excluded cases that are microsatellite-unstable or included all sporadic cases of colorectal cancer. Because cancers that originate along the serrated pathway tend to be microsatellite-unstable and represent only a subset of colorectal cancers (41), prior colorectal cancer GWAS may have missed many of the polymorphisms that are important in the serrated pathway. Therefore, new GWAS restricted to the subset of cancers most likely to arise from serrated polyps, specifically cancers that are microsatellite-unstable, carry a mutant v-Raf murine sarcoma viral oncogene homolog B gene (*BRAF*), and/or exhibit a CpG [cytosine-phosphate-guanine] island methylator phenotype (41), are need to identify genetic loci that are associated with the serrated pathway.

Strengths and limitations

This study included a large sample of well-characterized polyp cases and controls, with standard pathology review and complete ascertainment of case-control status via colonoscopy. However, our study's power to detect associations was limited because cases were divided into smaller subgroups based on the histological features of their polyps. This subgrouping was essential to evaluate possible heterogeneity in the association between different polyp groups and genetic variation in colorectal cancer susceptibility SNPs. In addition, our analyses involved comparisons between each SNP and each polyp type, and these multiple comparisons increased the likelihood of identifying spurious associations. However, we aimed to test the association between colorectal polyps and each SNP, not just to determine whether any of the colorectal cancer susceptibility SNPs were associated with polyps, and we have reported both statistically significant and nonsignificant associations. Finally, detection of SSPs is highly variable between clinicians (42). Therefore, these polyps may be more likely to have been missed at endoscopy, and some "polyp-free" controls may have actually harbored these lesions despite having had a complete colonoscopy. However, SSPs are relatively rare in the general population, with prevalence estimates ranging from 1% to 7% (9), so this possible misclassification is unlikely to have had a large impact on interpretation of the results.

Conclusions

Overall, our study suggests that the SNPs identified through colorectal cancer GWAS play a role in early carcinogenesis for the adenoma-carcinoma pathway. However, they do not appear to have a strong association with serrated lesions. These results support those of other studies suggesting different carcinogenic processes for the adenoma-carcinoma and serrated pathways to colorectal cancer. Additional GWAS aimed at identifying genetic loci associated with colorectal cancers that develop via the serrated pathway might shed light on the mechanisms which drive this subtype of colorectal cancer.

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