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Association of genetic variants for colorectal cancer differs by subtypes of polyps in the colorectum

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Most colorectal cancers originate from polyps, however, only a small proportion of polyps progress to carcinomas. Genome-wide association studies have identified multiple single-nucleotide polymorphisms (SNPs) in relation to colorectal cancer. Using these genetic risk variants, we evaluated whether colorectal cancer genetic factors may determine certain polyp phenotypes with different malignant potential. We analyzed 20 SNPs in 15 colorectal cancer susceptibility loci in a case–control study including 2473 cases (1831 with adenomas and 642 with hyperplastic polyps only) and 4019 controls. These patients were recruited from participants who received colonoscopy at two major hospitals in Nashville. A weighted genetic risk score (wGRS) was created to measure the cumulative association of multiple SNPs with polyp subtypes. Thirteen SNPs in 10 loci showed a statistically significant ($P < 0.05$, $n = 9$) or marginally significant ($P < 0.10$, $n = 4$) association with the risk of adenomas or hyperplastic polyps in the same direction as reported previously for colorectal cancer. A dose–response relation was observed between the wGRS and adenoma risk [per-allele odds ratio (OR) = 1.15, 95 confidence interval (CI): 1.10–1.20, $P_{trend} = 7.3 \times 10^{-10}$], with the association stronger for advanced than non-advanced adenomas ($P_{heterogeneity} = 0.038$), for multiple adenomas than a single adenoma ($P_{heterogeneity} = 0.039$), and for proximal than distal adenomas ($P_{heterogeneity} = 0.038$) and for adenomas diagnosed at younger than older age ($P_{heterogeneity} = 0.031$). A similar, but weak association between the wGRS and hyperplastic polyps was also observed (OR = 1.11, 95% CI: 1.04–1.18, $P_{trend} = 0.002$). These findings suggest that genetic factors play a significant role in the development of polyps with different malignant potential.

Introduction

Colorectal adenomatous polyps (adenomas) are well-established precursors of colorectal cancer (1,2), the second most common cause of cancer death in developed countries (3–6). Removal of adenomas has been shown to sharply reduce the mortality from colorectal cancer (7). In contrast, the link between hyperplastic polyps and cancer has been less clear. Recent data indicated that some hyperplastic polyps, particularly those with a large size may progress to colorectal cancer through perhaps the serrated neoplasia pathway (8–11). Therefore, some hyperplastic polyps may also be classified as cancer precursors.

At the somatic level, small polyps may progress to large and/or advanced polyps and eventually cancer through accumulation of mutations in key oncogenes and tumor suppressor genes involved in colorectal carcinogenesis (1,2). This adenoma–carcinoma sequence implies that the duration of tumorigenesis process may be important in determining the subtype of adenomas. We hypothesize that the subtype of colorectal polyps may also be determined by genetic factors that may affect the malignant potential of polyps. Specifically, we investigate whether individuals who are genetically at a high risk of colorectal cancer may be more likely to be diagnosed with advanced or multiple adenomas than a single small adenoma or hyperplastic polyps.

To test these hypotheses, we evaluated the association of subtypes of adenomas with 20 single-nucleotide polymorphisms (SNPs) that have been identified and robustly confirmed in recent genome-wide association studies (GWAS) to be associated with colorectal cancer (12–20). If the above-stated hypothesis is correct, we would expect to find that these colorectal cancer risk variants are more strongly related to multiple and/or advanced adenomas than a single small adenoma. We also investigated the association of these SNPs with the risk of hyperplastic polyps. Based on the hypothesis that some but not all hyperplastic polyps are precursors of colorectal cancer (10,11), we anticipate that cancer-related SNPs would be associated with the risk of hyperplastic polyps, but the strength of the association would be weaker than adenomas.

Abbreviations: CEU, Utah residents with Northern and Western European ancestry from the CEPH collection; CI, confidence interval; GWAS, genome-wide association studies; OR, odds ratio; QC, quality control; RSQ, R-squared; SNP, single-nucleotide polymorphism; wGRS, weighted genetic risk score.

Methods

Study populations

Participants for the current project were from the Tennessee Colorectal Polyp Study. The Tennessee Colorectal Polyp Study is a colonoscopy-based case–control study conducted in Nashville, TN and the details of this study have been described previously elsewhere (21,22). Briefly, participants for the study were recruited from those aged 40–75 years old, who were scheduled for colonoscopy at the Vanderbilt University Gastroenterology Clinic between February 2003 and October 2011 and the Veterans Affairs Tennessee Valley Health System Nashville Campus between August 2003 and May 2007. Of the 12 585 eligible individuals, 7621 (61%) agreed to participate in this study. Interviewers, who were blind to results of the colonoscopy, conducted a standardized telephone interview after the colonoscopy to obtain information from each participant regarding medication use, demographics, medical history, family history of cancer and polyps, reproductive history, anthropometry and selected lifestyle factors such as cigarette smoking, alcohol consumption and physical activity. A total of 6400 (84%) completed the interview. The relevant study protocols were approved by the Vanderbilt University Institutional Review Board, the Veterans' Affairs Tennessee Valley Health System Institutional Review Board and the Veterans' Affairs Tennessee Valley Health System Research & Development. All participants provided written informed consent.

Exclusion criteria were as follows: genetic colorectal cancer syndromes (i.e. hereditary non-polyposis colorectal cancer or familial adenomatous polyposis) or a prior history of inflammatory bowel disease, adenomas or any cancer other than non-melanoma skin cancer. Cases were participants diagnosed as any adenomas ($n = 2305$, including 522 participants who also had at least one hyperplastic polyp) and hyperplastic polyps only ($n = 767$) based on the colonoscopic examinations and pathologic findings. Controls were polyp-free participants ($n = 4340$) diagnosed using a complete colonoscopy reaching the cecum. Participants ($n = 209$) with other diagnoses were excluded from this study. Most of the participants (7443, 97.7% of all participants) donated a blood or exfoliated buccal cell sample or saliva to the study.

Adenomas were classified by size (<1 cm or ≥1 cm), histology (tubular, tubulovillous and villous), number (single or multiple) and location (proximal colon, distal colon, rectum or multiple locations) based on colonoscopy and pathology reports. An advanced adenoma was defined as an adenoma with 1 cm in size or larger, or tubulovillous or villous features or showing high-grade dysplasia or invasive cancer ($n = 45$).

Genotyping and imputation

The gene names used in this study were determined according to Human Gene Organization (http://www.hugo-international.org). We selected for this study 21

Table I. Selected characteristics of study participants by comparison groups, the Tennessee Colorectal Polyp Study, 2003–11

Variable	Controls (n = 4019)	Cases with colorectal polyps			
		Adenomas (n = 1831)		Hyperplastic polyps (n = 642)	
	No. (%)	No. (%)	P value[a]	No. (%)	P value[a]
Age (years, mean ± SD)	57.0 ± 7.7	58.8 ± 7.3	<0.001	56.9 ± 6.9	0.761
Sex, male	2286 (56.9)	1372 (74.9)	<0.001	445 (69.3)	<0.001
Race, white	3400 (84.6)	1564 (85.4)	0.417	563 (87.7)	0.041
Educational attainment					
High school or less	814 (23.9)	528 (33.8)	<0.001	189 (33.8)	<0.001
Some college	975 (28.6)	455 (29.2)		171 (30.5)	
College graduate	716 (21.0)	293 (18.8)		106 (18.9)	
Graduate or professional education	903 (26.5)	285 (18.3)		94 (16.8)	
Study site					
Academic medical center	2928 (72.9)	1061 (58.0)	<0.001	361 (56.3)	<0.001
Veterans affairs medical center	1090 (27.1)	769 (42.0)		280 (43.7)	
Indication for colonoscopy					
Screening	2256 (56.1)	1069 (58.4)	0.104	347 (54.1)	0.340
Other	1762 (43.9)	761 (41.6)		294 (45.9)	
Family history					
None	2414 (73.7)	1072 (72.6)	0.496	400 (74.1)	0.982
Colorectal polyps	581 (17.8)	264 (17.9)		94 (17.4)	
Colorectal cancer	279 (8.5)	141 (9.6)		46 (8.5)	
Body mass index (kg/m^2, mean ± SD)	28.1 ± 5.7	28.7 ± 5.6	<0.001		0.006
Use of non-steroidal anti-inflammatory drugs					
Never	1404 (40.8)	663 (41.8)	0.472	220 (38.9)	0.397
Ever	2041 (59.3)	922 (58.2)		346 (61.1)	
Cigarette smoking					
Never	1804 (52.5)	550 (34.8)	<0.001	155 (27.5)	<0.001
Former	1184 (34.5)	595 (37.6)		209 (37.1)	
Current	446 (13.0)	436 (27.6)		199 (35.6)	
Alcohol drinking					
Never	2033 (59.3)	780 (49.4)	<0.001	270 (48.0)	<0.001
Former	757 (22.1)	463 (29.3)		163 (29.0)	
Current	636 (18.6)	337 (21.3)		130 (23.1)	

[a]P value of comparison between cases and controls was derived from t-test or Mann–Whitney test for continuous variables and chi-square test or Fisher's exact test for categorical variables.

SNPs in 15 chromosomal loci that were identified in GWAS in relation to colorectal cancer risk (12–20) (Table II). Genomic DNA was extracted from buffy coats (*white blood cells*) or exfoliated buccal cells using QIAamp DNA kit (Qiagen, Valencia, CA) according to manufacturers' instructions. Saliva samples were collected using Oragene DNA sample collection kit (DNA Genotek, Kanata, Ontario, Canada) and DNA were extracted following manufacturer's protocol. Laboratory staff was blind to case–control status of the types of samples [study samples or quality control (QC) samples] during all experiments. Fourteen SNPs (rs16892766, rs10505477, rs10808555, rs6983267, rs7837328, rs7014346, rs10795668, rs3802842, rs4444235, rs4779584, rs9929218, rs4939827, rs10411210 and rs961253) were genotyped for all participants using the TaqMan OpenArray System (Applied Biosystems, Foster City, CA). Data for the remaining seven SNPs (rs6691170, rs6687758, rs10936599, rs7758229, rs7136702, rs11169552 and rs4925386) were obtained either by genotyping 4465 samples using Sequenom MassARRAY platform (Sequenom, San Diego, CA) or imputed using genotype data from the Affymetrix Genome-Wide Human SNP Array 5.0 (Affymetrix, Santa Clara, CA) for the remaining 2027 samples. We used MACH algorithm to impute genotypes for these SNPs since these SNPs are not included in the chip (23). All of these SNPs showed a high imputation quality with the RSQ, R-squared. values ranging from 0.84 to 1.00 with a mean value of 0.96. One SNP (rs11169552) failed in genotyping and thus was not included in the analysis. The average concordance of 217 paired samples for QC was 99.5% with a minimal rate of 96.2% for the SNPs genotyped by the TaqMan System. The concordance rate ranged from 98.8 to 100.0% with a mean rate of 99.4% for the 87 duplicate QC samples and was all greater than 95% with a mean rate of 98.2% for the 72 HapMap QC samples for the SNPs genotyped by the Sequenom platform. All 20 SNPs included in the current analysis were in Hardy–Weinberg equilibrium in controls ($P > 0.05$). None of the 20 SNPs were of multiple alleles.

Statistical analysis

The difference in descriptive characteristics between cases and controls was compared using Student's t-test or Mann–Whitney test for continuous variables and the chi-square test or Fisher's exact test for categorical variables. Test for Hardy–Weinberg equilibrium was performed for each SNP among controls using the Fisher's exact method to compare the expected and observed genotype distribution (24). Unconditional logistic regression models were used to estimate odds ratios (ORs) and 95% confidence intervals (CIs) with adjustment for age, sex, as well as risk factors such as body mass index, cigarette smoking and alcohol drinking. Per-allele ORs and 95% CIs were calculated based on the gene-dose effect model.

To determine the cumulative effect of genetic susceptibility variants, we constructed a weighted genetic risk score (wGRS) for each study participant by multiplying the number of risk alleles (0/1/2) of each SNP by the weight for that SNP, and then summing them together. For SNPs with a protective association for the minor allele, the major allele was treated as the risk allele in constructing the genetic score. The regression coefficient (β) or allelic OR (in log scale) reported from the initial GWAS was used as the weight to calculate the wGRS. When multiple GWAS reported the same SNP, we used the OR from the study with the largest sample size. For a locus in which multiple SNPs in linkage disequilibrium ($r^2 > 0.20$) were analyzed (i.e. five SNPs in 8q24 locus presented in Supplementary Table S1, available at *Carcinogenesis* Online), we selected the SNP with the most significant association (i.e. rs10505477 in 8q24 locus) to construct the wGRS. For the locus at 1q41, two SNPs (rs6691170 and rs6687758) were selected since these two SNPs are not in linkage disequilibrium ($r^2 = 0.131$ in CEU) and thus represent two independent signals as described in the initial GWAS (19). Therefore, 16 SNPs (rs6691170, rs6687758, rs10936599, rs7758229, rs16892766, rs10505477, rs10795668, rs3802842, rs7136702, rs4444235, rs4779584, rs9929218, rs4939827, rs10411210, rs961253 and rs4925386) were selected to construct the genetic score. On average, only 3% of participants had missing data for a particular SNP, and for these participants we assigned the median risk allele frequency of that SNP for cases or controls separately. We used mixed linear models to compare the mean wGRS between cases and controls. We next categorized wGRS into five groups according to the quintiles of the controls for risk estimate. We investigated the association by histologic types of colorectal polyps (any adenomas, hyperplastic polyps only and serrated adenomas), stage of adenomas (advanced or non-advanced adenomas), and multiplicity of

Table II. Association of GWAS-identified colorectal cancer risk variants with colorectal polyps risk in European Americans, the Tennessee Colorectal Polyp Study, 2003–11

SNP	Chromosome/gene	Allele[a]	Published GWAS[b]			All polyps (2127/3400)			Adenomas (1564)			Hyperplastic polyps (563)		
			OR_1	OR_2	OR_3	MAF[c]	OR (95% CI)[d]	*P* value	MAF[c]	OR (95% CI)[d]	*P* value	MAF[c]	OR (95% CI)[d]	*P* value
rs6691170	*1q41/DUSP10*	**T**/G	1.06	1.06	1.06	0.35	1.06 (0.98–1.15)	0.155	0.36	1.06 (0.97–1.16)	0.222	0.37	1.08 (0.94–1.23)	0.270
rs6687758	*1q41/DUSP10*	**G**/A	1.10	1.08	1.09	0.18	1.08 (0.98–1.19)	0.123	0.19	1.06 (0.95–1.19)	0.282	0.20	1.15 (0.98–1.35)	0.088
rs10936599	*3q26.2/MYNN*	**T**/C	0.91	0.95	0.93	0.25	0.93 (0.85–1.02)	0.136	0.25	0.97 (0.88–1.07)	0.582	0.23	0.84 (0.72–0.98)	0.024
rs7758229	*6q26-q27/SLC22A3*	**T**/G	1.46	NA	1.28	0.33	1.04 (0.96–1.13)	0.333	0.34	1.04 (0.95–1.14)	0.348	0.33	1.03 (0.90–1.18)	0.625
rs16892766	*8q23/EIF3H*	**C**/A	NA	NA	1.25	0.07	1.05 (0.91–1.22)	0.510	0.08	1.06 (0.90–1.25)	0.464	0.07	0.98 (0.77–1.26)	0.892
rs10505477	*8q24/MYC*	**A**/G	1.22	NA	1.17	0.50	1.13 (1.04–1.22)	0.003	0.52	1.13 (1.03–1.23)	0.008	0.53	1.13 (0.99–1.29)	0.063
rs10808555	*8q24/MYC*	**G**/A	NA	NA	1.16	0.34	1.04 (0.95–1.13)	0.407	0.35	1.05 (0.95–1.15)	0.345	0.34	1.01 (0.88–1.16)	0.886
rs6983267	*8q24/MYC*	**G**/T	1.43	NA	1.21	0.51	1.11 (1.02–1.20)	0.012	0.53	1.12 (1.03–1.23)	0.010	0.53	1.07 (0.94–1.22)	0.281
rs7837328	*8q24/MYC*	**A**/G	1.23	NA	1.17	0.41	1.10 (1.01–1.19)	0.023	0.43	1.11 (1.02–1.21)	0.021	0.42	1.07 (0.94–1.22)	0.308
rs7014346	*8q24/MYC*	**A**/G	1.20	1.19	1.19	0.36	1.08 (0.99–1.17)	0.067	0.38	1.09 (0.99–1.19)	0.070	0.38	1.06 (0.93–1.21)	0.377
rs10795668	*10p14/FLJ3802842*	**A**/G	NA	NA	0.89	0.32	0.95 (0.87–1.04)	0.260	0.30	0.92 (0.83–1.01)	0.076	0.34	1.06 (0.92–1.23)	0.385
rs3802842	*11q23*/unknown	**C**/A	1.20	1.09	1.11	0.29	1.06 (0.97–1.15)	0.204	0.30	1.07 (0.98–1.18)	0.148	0.29	1.02 (0.89–1.17)	0.797
rs7136702	*12q13.13/LARP4,DIP2*	**T**/C	1.06	1.05	1.06	0.34	1.01 (0.93–1.09)	0.830	0.35	1.04 (0.95–1.14)	0.345	0.32	0.91 (0.80–1.04)	0.175
rs4444235	*14q22/BMP4*	**C**/T	1.12	1.10	1.11	0.46	1.07 (0.99–1.15)	0.103	0.47	1.04 (0.96–1.14)	0.343	0.49	1.13 (1.00–1.29)	0.057
rs4779584	*15q13/GREM1*	**T**/C	1.35	NA	1.26	0.18	1.19 (1.08–1.32)	$4.1\times10-4$	0.20	1.18 (1.06–1.31)	0.003	0.21	1.23 (1.05–1.44)	0.010
rs9929218	*16q22/CDH1*	**A**/G	0.88	0.93	0.91	0.29	0.93 (0.85–1.01)	0.083	0.27	0.90 (0.82–1.00)	0.042	0.30	1.00 (0.87–1.15)	0.987
rs4939827	*18q21/SMAD7*	**C**/T	0.71	NA	0.85	0.48	0.90 (0.83–0.98)	0.014	0.45	0.88 (0.81–0.97)	0.007	0.47	0.95 (0.83–1.08)	0.447
rs10411210	*19q13/RHPN2*	**T**/C	0.79	0.90	0.87	0.11	0.86 (0.75–0.98)	0.024	0.10	0.93 (0.81–1.07)	0.321	0.08	0.68 (0.54–0.86)	0.001
rs961253	*20p12/BMP2*	**A**/C	1.13	1.11	1.12	0.36	0.97 (0.89–1.06)	0.504	0.36	1.00 (0.91–1.10)	0.972	0.34	0.89 (0.77–1.03)	0.113
rs4925386	*20q13.33/LAMA5*	**T**/C	0.93	0.93	0.93	0.30	0.87 (0.80–0.95)	0.002	0.25	0.80 (0.72–0.88)	5.7×10^{-6}	0.31	1.11 (0.96–1.27)	0.156

[a]Minor/major alleles as reported from GWAS of colorectal cancer. All results based on the minor allele (bold) of the SNP evaluated.
[b]ORs, including OR_1 (discovery stage), OR_2 (replication stage) and OR_3 (overall) from the original studies; NA, not available.
[c]Minor allele frequency of controls in overall and of cases in adenomas and hyperplastic polyps.
[d]Adjusted for age, sex, body mass index, cigarette smoking and alcohol drinking.

adenomas (single or multiple), adenoma location (proximal colon, distal colon or rectum) and age at index colonoscopy (<50, 50–65 and >65 years). Test for trend across comparison groups was performed using the wGRS as continuous variable. We use Cochran's *Q* statistic to test for heterogeneity across different comparison groups (25).

Statistical analyses were performed with the use of SAS software, version 9.3 (SAS Institute, Cary, NC). Unless stated otherwise, two-sided *P* values of less than 0.05 were considered to indicate statistical significance.

Results

Characteristics of this study

A total of 2473 cases (1831 participants with any adenomas and 642 with hyperplastic polyps only) and 4019 polyp-free controls were included in this analysis. Of them, 2127 cases (1564 participants with any adenomas and 563 with hyperplastic polyps only) and 3400 controls were European Americans and 260 cases (203 participants with any adenomas) and 461 controls were African Americans. Selected characteristics of cases and controls are presented in Table I. Traditional risk factors including male sex, high body mass index, cigarette smoking and alcohol drinking were found to be significantly associated with colorectal polyps ($P < 0.05$). Low level of education was also significantly related to the risk of polyps ($P < 0.001$). Age was a strong risk factor for adenomas ($P < 0.001$) but not for hyperplastic polyps. No significant association was found between polyp risk with a family history of colorectal cancer or polyps. Ever use of non-steroidal anti-inflammatory drugs was associated with a reduced risk of adenomas after adjusting for age and sex ($P < 0.001$) although this association was not statistically significant in the unadjusted analysis.

Associations of colorectal polyp risk with individual SNPs

Among European Americans (Table II), statistically significant associations with all polyps combined were found for 7 (rs10505477, rs6983267, rs7837328, rs4779584, rs4939827, rs10411210 and rs4925386) of the 20 GWAS-identified colorectal cancer risk variants ($P < 0.05$). Two additional variants (rs7014346 and rs9929218) showed marginally significant associations ($P < 0.10$). With the exception of rs962153, the ORs for the remaining 10 SNPs were also in the same direction as reported previously for colorectal cancer. Virtually all but two (rs10795668 and rs10411210) of the SNPs that showed a statistically or marginally significant association with all polyps combined were also associated with the risk of any adenomas. When these analyses were performed for the hyperplastic polyps only group, three SNPs (rs10936599, rs4779584 and rs10411210) showed a significant association ($P < 0.05$) and three additional SNPs (rs6687758, rs10505477 and rs4444235) had a marginally significant association ($P < 0.10$). Heterogeneity between adenomas and hyperplastic polyps was found to be statistically significant ($P < 0.05$) for two SNPs (rs10411210 and rs4925386) and marginally significant ($P < 0.10$) for two additional SNPs (rs10795668 and rs7136702) (data not shown in tables). Of the nine SNPs that were either statistically or marginally significant associated with the risk of adenomas, most of them showed a stronger association with multiple adenomas than a single adenoma (Table III). A similar finding was seen for the comparison between advanced than non-advanced adenomas (Table III). Analyses in African Americans revealed a statistically significant association for one SNP (rs6983267, OR = 1.63, 95% CI: 1.09–2.44; $P = 0.017$) and a marginally significant association ($P < 0.10$) for two additional SNPs (rs10505477 and rs10795668). Because of a small sample size, detailed results for African Americans are not reported in this paper.

Associations of colorectal polyp risk with the genetic risk score

Table IV presents the association of the wGRS including 16 independent SNPs with the risk of polyps among European Americans. In the multivariable adjusted model, a dose–response relation between the wGRS and polyp risk was observed for both adenomas (top versus bottom quintile of wGRS: OR = 1.86, 95% CI: 1.51–2.27; $P_{trend} = 7.3 \times 10^{-10}$) and hyperplastic polyps only (OR = 1.47, 95% CI: 1.10–1.96; $P_{trend} = 0.002$). A stronger association was noted for adenomas than hyperplastic polyps only although the test for heterogeneity was not statistically significant ($P_{heterogeneity} = 0.374$), perhaps due to a small sample size in the hyperplastic polyps only group. Among the adenomas group, the association was stronger for advanced than non-advanced adenomas ($P_{heterogeneity} = 0.038$) and multiple adenomas than a single adenoma ($P_{heterogeneity} = 0.039$). The association

Table III. Association of GWAS-identified colorectal cancer risk variants and adenomas by subgroups in European Americans, the Tennessee Colorectal Polyp Study, 2003–11

SNP	Allele[a]	Non-advanced versus advanced					Single versus multiple				
		Non-advanced adenomas		Advanced adenomas		$P_{heterogeneity}$	Single adenoma		Multiple adenomas		$P_{heterogeneity}$
		OR (95% CI)[b]	*P* value	OR (95% CI)[b]	*P* value		OR (95% CI)[b]	*P* value	OR (95% CI)[b]	*P* value	
rs6691170	**T**/G	1.06 (0.96–1.17)	0.288	1.06 (0.91–1.24)	0.440	0.941	1.07 (0.96–1.19)	0.221	0.95 (0.81–1.10)	0.470	0.194
rs6687758	**G**/A	1.06 (0.94–1.20)	0.357	1.06 (0.87–1.28)	0.556	0.996	1.09 (0.96–1.25)	0.189	0.90 (0.74–1.09)	0.273	0.100
rs10936599	**T**/C	0.98 (0.88–1.10)	0.736	0.93 (0.78–1.11)	0.407	0.602	1.06 (0.94–1.19)	0.375	0.88 (0.74–1.04)	0.138	0.085
rs7758229	**T**/G	1.04 (0.94–1.16)	0.399	1.10 (0.94–1.28)	0.244	0.609	1.08 (0.97–1.21)	0.157	1.01 (0.86–1.17)	0.936	0.446
rs16892766	**C**/A	1.00 (0.84–1.21)	0.963	1.19 (0.92–1.55)	0.195	0.299	1.09 (0.90–1.32)	0.386	1.00 (0.76–1.31)	0.978	0.600
rs10505477	**A**/G	1.07 (0.97–1.18)	0.194	1.34 (1.15–1.55)	1.7×10^{-4}	0.014	1.05 (0.94–1.16)	0.411	1.28 (1.11–1.48)	0.001	0.028
rs10808555	**G**/A	1.03 (0.93–1.14)	0.570	1.10 (0.94–1.28)	0.251	0.513	1.02 (0.91–1.14)	0.744	1.07 (0.92–1.25)	0.371	0.600
rs6983267	**G**/T	1.07 (0.97–1.18)	0.181	1.32 (1.13–1.53)	4.3×10^{-4}	0.025	1.05 (0.95–1.17)	0.336	1.26 (1.09–1.46)	0.002	0.051
rs7837328	**A**/G	1.06 (0.96–1.17)	0.269	1.29 (1.11–1.50)	0.001	0.030	1.07 (0.96–1.19)	0.203	1.22 (1.05–1.41)	0.007	0.163
rs7014346	**A**/G	1.05 (0.95–1.16)	0.382	1.22 (1.05–1.43)	0.010	0.093	1.04 (0.94–1.17)	0.442	1.20 (1.03–1.39)	0.017	0.146
rs10795668	**A**/G	0.92 (0.82–1.02)	0.119	0.92 (0.78–1.09)	0.325	0.988	0.90 (0.80–1.02)	0.095	0.98 (0.83–1.14)	0.760	0.457
rs3802842	**C**/A	1.04 (0.94–1.16)	0.437	1.15 (0.98–1.35)	0.092	0.325	1.03 (0.91–1.15)	0.670	1.14 (0.98–1.33)	0.100	0.288
rs7136702	**T**/C	1.05 (0.95–1.16)	0.318	1.03 (0.88–1.21)	0.685	0.841	1.05 (0.94–1.17)	0.361	1.07 (0.92–1.24)	0.392	0.876
rs4444235	**C**/T	1.03 (0.94–1.14)	0.517	1.08 (0.93–1.25)	0.342	0.654	1.01 (0.91–1.12)	0.828	1.05 (0.91–1.21)	0.525	0.698
rs4779584	**T**/C	1.15 (1.02–1.30)	0.020	1.22 (1.02–1.47)	0.029	0.592	1.08 (0.95–1.24)	0.248	1.26 (1.06–1.50)	0.009	0.169
rs9929218	**A**/G	0.94 (0.84–1.04)	0.246	0.79 (0.67–0.94)	0.009	0.107	0.91 (0.80–1.02)	0.097	0.93 (0.79–1.09)	0.360	0.811
rs4939827	**C**/T	0.86 (0.78–0.95)	0.003	0.95 (0.82–1.11)	0.545	0.272	0.91 (0.81–1.01)	0.074	0.88 (0.76–1.02)	0.089	0.754
rs10411210	**T**/C	0.91 (0.78–1.07)	0.247	0.98 (0.77–1.25)	0.882	0.610	1.03 (0.88–1.22)	0.695	0.82 (0.64–1.05)	0.121	0.131
rs961253	**A**/C	0.99 (0.89–1.11)	0.916	1.02 (0.86–1.20)	0.857	0.834	0.98 (0.87–1.11)	0.770	1.03 (0.88–1.21)	0.693	0.623
rs4925386	**T**/C	0.80 (0.72–0.90)	1.1 × 10–4	0.77 (0.65–0.92)	0.004	0.703	0.80 (0.71–0.90)	3.0×10^{-4}	0.75 (0.63–0.89)	0.001	0.516

[a]Minor/major allele as reported from GWAS of colorectal cancer. All results based on the minor allele (bold) of the SNP evaluated.
[b]Adjusted for age, sex, body mass index, cigarette smoking and alcohol drinking.

Table IV. Cumulative association of the weighted genotype risk score on variable phenotypes of colorectal polyps in European Americans, the Tennessee Colorectal Polyp Study, 2003–11

Type of colorectal polyps	No. of cases	OR (95% CI), by quintile of weighted genetype risk score[a]					Per-allele association[a]		$P_{heterogeneity}$
		Q1 (low)	Q2	Q3	Q4	Q5	OR (95% CI)	P_{trend}	
Overall	2127	Reference	1.19 (0.98–1.43)	1.21 (1.00–1.46)	1.41 (1.17–1.69)	1.75 (1.46–2.10)	1.14 (1.10–1.19)	1.5×10^{-10}	
Any adenomas	1564	Reference	1.28 (1.04–1.58)	1.20 (0.97–1.48)	1.47 (1.20–1.80)	1.86 (1.52–2.27)	1.15 (1.10–1.20)	7.3×10^{-10}	0.341
Hyperplastic polyps only	563	Reference	0.96 (0.70–1.31)	1.23 (0.91–1.65)	1.22 (0.91–1.64)	1.47 (1.10–1.96)	1.11 (1.04–1.18)	0.002	
Adenomas									
Non-advanced adenomas	1152	Reference	1.33 (1.06–1.67)	1.15 (0.91–1.45)	1.47 (1.18–1.84)	1.73 (1.39–2.16)	1.13 (1.07–1.18)	1.6×10^{-6}	0.038
Advanced adenomas	394	Reference	1.17 (0.79–1.71)	1.45 (1.00–2.11)	1.48 (1.02–2.13)	2.29 (1.62–3.23)	1.22 (1.13–1.32)	6.7×10^{-7}	
Single adenoma	908	Reference	1.28 (1.00–1.64)	1.11 (0.86–1.42)	1.41 (1.11–1.80)	1.58 (1.24–2.00)	1.11 (1.05–1.17)	1.7×10^{-4}	0.039
Multiple adenomas	435	Reference	1.24 (0.86–1.78)	1.29 (0.90–1.84)	1.57 (1.11–2.21)	2.05 (1.46–2.86)	1.19 (1.10–1.28)	7.5×10^{-6}	
Single small adenoma	715	Reference	1.30 (1.00–1.71)	1.06 (0.80–1.41)	1.45 (1.12–1.89)	1.54 (1.19–2.01)	1.10 (1.04–1.17)	9.3×10^{-4}	0.005
Advanced and multiple adenomas	150	Reference	1.07 (0.57–2.02)	1.52 (0.84–2.73)	1.36 (0.75–2.47)	2.73 (1.60–4.67)	1.28 (1.13–1.45)	7.0×10^{-5}	
Distal adenomas	522	Reference	1.31 (0.96–1.78)	1.03 (0.74–1.42)	1.29 (0.95–1.75)	1.54 (1.14–2.08)	1.09 (1.02–1.17)	0.012	0.038
Any proximal adenomas	882	Reference	1.24 (0.95–1.61)	1.27 (0.98–1.65)	1.49 (1.15–1.92)	2.08 (1.63–2.66)	1.19 (1.12–1.25)	1.3×10^{-9}	
Distal adenomas	522	Reference	1.31 (0.96–1.78)	1.03 (0.74–1.42)	1.29 (0.95–1.75)	1.54 (1.14–2.08)	1.09 (1.02–1.17)	0.012	0.001
Advanced proximal adenomas	229	Reference	1.05 (0.61–1.80)	1.82 (1.12–2.96)	1.55 (0.94–2.53)	2.93 (1.86–4.61)	1.31 (1.18–1.45)	2.2×10^{-7}	
Age <50 years	101	Reference	1.14 (0.56–2.35)	1.13 (0.55–2.34)	1.10 (0.53–2.28)	2.69 (1.44–5.03)	1.27 (1.10–1.47)	0.001	0.031
Age 50–65 years	1132	Reference	1.28 (1.01–1.62)	1.20 (0.95–1.53)	1.58 (1.26–1.99)	1.87 (1.50–2.35)	1.16 (1.10–1.22)	6.2×10^{-9}	
Age >65 years	331	Reference	1.42 (0.97–2.08)	1.23 (0.84–1.82)	1.31 (0.90–1.92)	1.58 (1.09–2.28)	1.08 (1.00–1.18)	0.050	

[a]Sixteen SNPs were used to construct the score, including rs6691170, rs6687758, rs10936599, rs7758229, rs16892766, rs10505477, rs10795668, rs3802842, rs7136702, rs4444235, rs4779584, rs9929218, rs4939827, rs10411210, rs961253 and rs4925386. Results were adjusted for age (except for the analysis stratified by age), sex, body mass index, cigarette smoking and alcohol drinking.

was much stronger for the group with both advanced and multiple adenomas (OR = 2.73, 95% CI: 1.60–4.67, for the top versus bottom quintile of wGRS; $P_{trend} = 7.0\times10^{-5}$) than those only with a single small adenoma (OR = 1.54, 95% CI: 1.19–2.01; $P_{trend} = 9.3\times10^{-4}$) ($P_{heterogeneity} = 0.005$). Furthermore, when stratified by tumor location, the association between the wGRS and adenoma risk was stronger for those in the proximal (OR = 2.08, 95% CI: 1.63–2.66, for the top versus bottom quintile of wGRS; $P_{trend} = 1.3\times10^{-9}$) than those in the distal colon (OR = 1.54, 95% CI: 1.14–2.08; $P_{trend} = 0.012$) ($P_{heterogeneity} = 0.038$) and much higher risk estimates were observed particularly for participants with advanced adenomas in the proximal colon (OR = 2.93, 95% CI: 1.86–4.61, for the top versus bottom quintile of wGRS; $P_{trend} = 2.2\times10^{-7}$) than those in the distal colon ($P_{heterogeneity} = 0.001$). When stratified by age, the association of the wGRS with adenomas was found to be stronger among participants younger than 50 years of age (OR = 2.69, 95% CI: 1.44–5.03, for the top versus quintile bottom of wGRS; $P_{trend} = 0.001$) than those older than aged 65 years (OR = 1.58, 95% CI: 1.09–2.28; $P_{trend} = 0.050$) ($P_{heterogeneity} = 0.031$).

Discussion

In this large case–control study involving around 6500 participants, we have shown that most of the GWAS-identified genetic risk variants for colorectal cancer were also associated with the risk of adenomas or hyperplastic polyps. The strength of the association, however, differed substantially by adenoma characteristics. In particular, cancer-related genetic variants were more closely related to the risk of developing multiple or advanced adenomas, particularly at young age or in the proximal colon than other polyps. These results suggest that at least a portion of the adenoma's characteristics is predetermined by genetic factors and individuals who are diagnosed with the above-mentioned adenomas may be more likely to develop colorectal cancer than those with other polyps. Identification of genetic factors that are differentially associated with the adenoma subtypes may help elucidate biological mechanisms for the initiation and progression of colorectal tumors.

Several previous studies have evaluated the association of GWAS-identified colorectal cancer variants with adenoma risk (13,14,26,27). Several of the colorectal-cancer-related SNPs, including rs6983267, rs4939827 and rs3802842, have been shown to be related to adenoma risk (13,14,26,27). However, most colorectal-cancer-related SNPs have not yet been reported or even evaluated in relation to polyp risk. Furthermore, most of the previous studies had a small sample size, which may explain the lack of statistical associations observed in those studies. The current study represents the most systematic evaluation to date for the association of colorectal-cancer-related SNPs with adenoma risk. This study reported, for the first time, several associations between polyp risk and colorectal-cancer-related SNPs, including rs6687758, rs10936599, rs16892766, rs10505477, rs7837328, rs4779854, rs9929218, rs10411210 and rs4925386. Furthermore, this is the first study that systematically evaluated the association of colorectal-cancer-related SNPs with the risk of polyps by subtypes to test the hypothesis that some of the polyp characteristics may be predetermined by genetic factors and participants who developed colorectal cancer may be genetically more similar to those who developed advanced or multiple adenomas than those who developed a single non-advanced adenoma.

In agreement with our hypothesis, we found that the wGRS constructed using colorectal-cancer-related SNPs was more closely associated with the risk of multiple adenomas than a single adenoma. This finding is supported by several previous studies. A family history of colorectal cancer has been shown to be related more closely with multiple adenomas than a single adenoma (28). The influence of genetic risk factors on adenoma multiplicity has been observed in patients with a Mendelian predisposition to colorectal tumors (29,30) including familial adenomatous polyposis, Peutz-Jeghers syndrome and juvenile polyposis syndrome. However, very few studies have examined this phenomenon in sporadic adenomas. A recent pooled analysis showed that the association for both rs6983267 and the haplotype

including this SNP in 8q24 was more strongly associated with multiple adenomas than a single adenoma with statistically significant heterogeneity (26).

To date, little evidence exists to support the notion that genetic factors may play a more significant role in the risk of developing advanced adenomas than non-advanced adenomas. It remains elusive whether individuals with familial and hereditary colorectal cancer syndromes are more likely to develop advanced adenomas (29,30). To our knowledge, only one study has examined the association of colorectal-cancer-related SNPs with the risk of advanced and non-advanced adenomas; however, that study did not identify any significant heterogeneity between these two groups of adenomas (26). Instead of using individual SNPs, we used wGRS, a measurement of the cumulative effect of multiple SNPs, in the analysis and found a stronger association of this score with advanced than non-advanced adenomas. A recent study of colorectal cancer provided some support to our findings. In that study, rs16892766 in 8q23 locus was shown to be more closely related to aggressive cancer phenotypes than low grade cancer (31).

It has been hypothesized that the genetic contribution to the occurrence of adenomas and cancer in the proximal colon may be larger than those in rectum and distal colon (32,33). This hypothesis has been supported by the finding that more neoplasia in the proximal colon than in the distal colon and rectum have microsatellite instability, a phenotype that is caused to a large extent by mismatch gene mutation or methylation (34). It has been clearly demonstrated that germline mutations of DNA mismatch repair genes are responsible for the Lynch syndrome (hereditary non-polyposis colorectal cancer), and individuals with this syndrome are more likely to have cancer in the proximal colon than do individuals in the general population (29,30). In this study, we demonstrated for the first time that common genetic variants that are not in the DNA mismatch repair genes are also more closely related to neoplasia in the proximal colon than neoplasia in the distal colon.

Individuals with a familial cancer syndrome, including familial adenomatous polyposis and hereditary non-polyposis colorectal cancer, are much more likely to have an early-onset cancer than the general population (29,30). It remains unclear, however, if common genetic risk variants may be related to an elevated risk of early-onset cancer. We found in this study that the wGRS was more closely related to adenomas diagnosed at a younger than older age, providing some support that common genetic risk variants could contribute to early onset of colorectal cancer.

To our knowledge, no study has evaluated the association of GWAS-identified colorectal cancer genetic variants with the risk of hyperplastic polyps. Because of a small sample size for hyperplastic polyps, our study did not have adequate power to evaluate the association of individual SNPs with the risk of this polyp group. Nevertheless, three of the SNPs evaluated showed a statistically significant association. More important, a dose–response relationship between the wGRS and hyperplastic polyps was observed, suggesting that this group of polyps share some genetic risk factors with colorectal cancer. The finding that the association of the wGRS is weaker for hyperplastic polyps than adenomas suggests that genetically individuals who had an adenoma are more likely to develop colorectal cancer than those who had hyperplastic polyps.

This is the largest study conducted to date to evaluate GWAS-identified colorectal-cancer-related SNPs with the risk of polyps by subtypes. Unlike most previous studies that evaluated only a few SNPs, we systematically evaluated all colorectal-cancer-related SNPs reported to date. Colonoscopy was used in the study to define case–control status and classify case subgroups so that potential misclassification among study groups was minimized. We also collected extensive exposure data allowing us to adjust carefully for potential confounding factors. Despite an overall large sample size for the study, the number of cases for hyperplastic polyps only, remain small, which has affected the statistical power for evaluating the association of individual SNPs and performing formal tests for potential difference in wGRS association between this polyp group and other polyp groups. Nevertheless, this study represents one of the largest investigations conducted to date for genetic risk factors for hyperplastic polyps. For six SNPs, we used imputation data from GWAS for 2027 subjects, approximately 30% of the total sample size included in the current analysis. Imputation dosage data were used to account for imputation uncertainty as typically conducted in genetic studies using imputation data (35). The results were virtually unchanged after excluding these subjects from the study, which is not surprising given the high imputation quality of these data. In a subset of subjects (n = 145) who were included in the GWAS and targeted genotyping, we found that the mean concordance between imputation and genotyping data was 97.1%.

In summary, we found that GWAS-identified colorectal cancer SNPs, in aggregate, are more strongly related to multiple adenomas than single adenoma, advanced than non-advanced adenomas and proximal adenomas than distal adenomas. In other words, part of the adenoma's characteristics may be predetermined by genetic factors, and individuals who are found to have multiple, advanced and/or proximal adenomas may be more likely to develop colorectal cancer than those who are diagnosed with other polyps. In addition, we found that genetic risk variants for colorectal cancer also were associated with the risk of hyperplastic polyps, supporting the notion that some hyperplastic polyps are precursors of colorectal cancer. These findings may have significant implication for identifying high-risk patients for cancer screening and chemoprevention and for future studies to investigate genetic and biological basis for the initiation and progression of colorectal tumors.

Supplementary material

Supplementary Table S1 can be found at http://carcin.oxfordjournals.org/

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