

Germline Variants and Advanced Colorectal Adenomas: Adenoma Prevention with Celecoxib Trial Genome-wide Association Study

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Abstract

Purpose: Identification of single-nucleotide polymorphisms (SNP) associated with development of advanced colorectal adenomas.

Experimental Design: Discovery phase: 1,406 Caucasian patients (139 advanced adenoma cases and 1,267 controls) from the Adenoma Prevention with Celecoxib (APC) trial were included in a genome-wide association study (GWAS) to identify variants associated with postpolypectomy disease recurrence. Genome-wide significance was defined as false discovery rate less than 0.05, unadjusted $P = 7.4 \times 10^{-7}$. Validation phase: results were further evaluated using 4,175 familial colorectal adenoma cases and 5,036 controls from patients of European ancestry [COLOrectal Gene Identification consortium (CORGI), Scotland, Australia, and VQ58].

Results: Our study identified eight SNPs associated with advanced-adenoma risk in the APC trial (rs2837156, rs7278863, rs2837237, rs2837241, rs2837254, rs741864 at 21q22.2, and rs1381392 and rs17651822 at 3p24.1, at $P < 10^{-7}$ level with $OR > 2$). Five variants in strong pairwise linkage disequilibrium (rs7278863, rs2837237, rs741864, rs741864, and rs2837241; $r^2 = 0.8-1$) are in or near the coding region for the tight junction adhesion protein, IGSF5. An additional variant associated with advanced adenomas, rs1535989 [minor allele frequency, 0.11; OR , 2.09; 95% confidence interval (CI), 1.50–2.91], also predicted colorectal cancer development in a validation analysis ($P = 0.019$) using a series of adenoma cases or colorectal cancer (CORGI study) and 3 sets of colorectal cancer cases and controls (Scotland, VQ58, and Australia; $N = 9,211$).

Conclusions: Our results suggest that common polymorphisms contribute to the risk of developing advanced adenomas and might also contribute to the risk of developing colorectal cancer. The variant at rs1535989 may identify patients whose risk for neoplasia warrants increased colonoscopic surveillance. *Clin Cancer Res*; 19(23); 6430–7. ©2013 AACR.

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Note: Supplementary data for this article are available at Clinical Cancer Research Online (<http://clincancerres.aacrjournals.org/>).

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Introduction

Colorectal cancer is a common malignancy, with prevalence in developed nations of 40 to 50 cases per 100,000 individuals (1). Approximately one-third of those diagnosed with colorectal cancer will die of their disease due to diagnosis at a stage not curable by locoregional therapy. Most colorectal cancer cases arise from premalignant adenomas that require years or even decades to progress to invasive disease. Colonoscopy to identify and remove precursor adenomas has been recommended for more than 25 years for patients at high colorectal cancer risk, and recently completed long-term analyses of screened cohorts confirmed the utility of adenoma removal for preventing deaths due to colorectal cancer (2).

Our goal is to understand the biology of colorectal cancer to develop effective prevention and therapy, and also to characterize individual risk in a manner that will identify patients most likely to benefit from colonoscopy to detect

Translational Relevance

Identification of patients at highest risk of colorectal cancer is essential for providing optimal disease screening and prevention. This study uncovers germline susceptibility loci that indicate risk of disease and potential for improved understanding of disease biology.

and remove premalignant adenomas. Identification of germline variants conveying an increased risk of colorectal cancer could be used to promote adherence to colonoscopy and polypectomy for patients at highest risk, improving utilization and cost-benefit of this life-saving procedure. In addition, more accurate characterization of high-risk individuals would facilitate participation in prevention clinical trials. Finally, therapy to treat or prevent colorectal cancer would be advanced if the biologic consequences of germline susceptibility variants were further characterized to uncover the molecular basis of colorectal cancer.

The adenoma–carcinoma sequence in the colorectum represents a disease spectrum. Adenomas with a low risk of cancer development are small (<0.6 cm diameter) and lack histologic features associated with progression, such as the presence of villous features or high-grade dysplasia. The identification of an advanced adenoma (size ≥ 1 cm; villous or tubulovillous histology; high-grade dysplasia) indicates that a patient has a higher risk of future adenoma and colorectal cancer development (3). Advanced adenomas, therefore, are the most important lesions to target for colorectal cancer prevention. In this study, we used a large cohort of patients with adenoma from a prospective randomized clinical trial to identify single-nucleotide polymorphisms (SNP) associated with increased risk of developing advanced adenomas. These variants were then further tested using large genotyped cohorts of patients and controls with advanced adenomas and colorectal cancer. In doing so, we identified variants associated with both advanced premalignant lesions and colorectal cancer.

Materials and Methods

Study design and populations

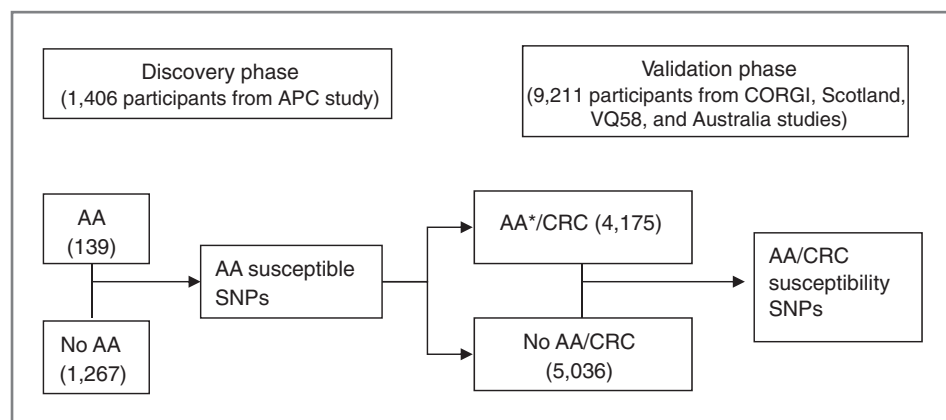
The overall study design is illustrated in Fig. 1.

Discovery phase. Of note, 1,406 evaluable Caucasian patients were identified from the Adenoma Prevention with Celecoxib (APC) trial, a randomized, placebo-controlled study to test whether celecoxib reduced the occurrence of endoscopically detected colorectal adenomas. The endpoint advanced adenoma was defined as any adenoma with size 1 cm or more, villous/tubulovillous histology, or high-grade dysplasia. During the prospective follow-up period, 139 participants developed advanced adenomas identified during a scheduled colonoscopy screening examination. Detailed information about the trial design and primary outcomes was reported elsewhere (4).

Validation phase. The advanced adenoma susceptibility that SNPs identified from APC trial were further evaluated using genome-wide association study (GWAS) data from the following four nonoverlapping colorectal cancer case-control series of European ancestry (5).

1. CORGI: 931 familial colorectal adenoma or colorectal cancer cases and 929 cancer-free controls of White British origin ascertained through the COloRectal Gene Identification (CORGI) consortium. All cases had at least one first-degree relative with colorectal tumors and no mutations in the known highly penetrant colorectal cancer genes. Controls were spouses or partners of the cases and had no personal history of colorectal cancer (6).
2. Scotland: 1,003 early-onset Scottish colorectal cancer cases (<55 years) and 979 cancer-free Scottish population controls. Known Mendelian syndromes were excluded. Controls were matched by age (± 5 years), gender, and area of residence (6).
3. VQ58: 1,800 British Stage II/III patients with colorectal cancer from the VICTOR ($N = 923$) and QUASAR2 (<http://www.octo-oxford.org.uk/alltrials/trials/q2.html>; $N = 877$) clinical trials, together with publicly available data from 2,690 population controls from the Wellcome Trust Case–Control Consortium (WTCCC) 1958 Birth Cohort (7).

Figure 1. Study design. *Including subjects with familial adenoma from CORGI. AA, advanced adenoma; CRC, colorectal cancer.



4. Australia: 441 colorectal cancer cases treated in the Royal Melbourne, Western and St Francis Xavier Cabrini Hospitals in Melbourne and 438 population controls from Brisbane Twin Nevus and Genes in Myopia studies, matched to the cases using principal component analysis (6).

Thus, 4,175 familial colorectal adenoma or colorectal cancer cases and 5,036 controls were included in the validation analysis. Human Subjects Committee approval to collect and genotype whole blood samples was obtained by Brigham and Women's Hospital and the RIKEN Center for Genomic Medicine.

Genotyping and quality control

DNA was isolated from blood samples using standard methods and quantified with picogreen. For the APC cohort, genotyping was performed by the RIKEN Center

for Genomic Medicine using the Illumina Human610-Quad BeadChip platform (Illumina). A White parent-child Centre d'Etude du Polymorphisme Humain (CEPH) trio from the HapMap was used to check for Mendelian transmission of alleles. χ^2 test based on genotype frequencies at each SNP was used to test for deviations from Hardy-Weinberg equilibrium (HWE). Any SNP with HWE $P < 0.001$ was excluded. Two cases and two controls were randomly chosen as duplicates for quality control of genotype concordance. A total of 28 subjects and 1,792 markers were excluded for quality control reasons, including duplicates, those that showed identity-by-descent more than 12.5% or were gender mismatched, samples with less than 98%, and markers with less than 99% call rate or heterozygous haploids. The final Manhattan plot and QQ plot indicated the satisfactory quality control process (Supplementary Figs. S1 and S2).

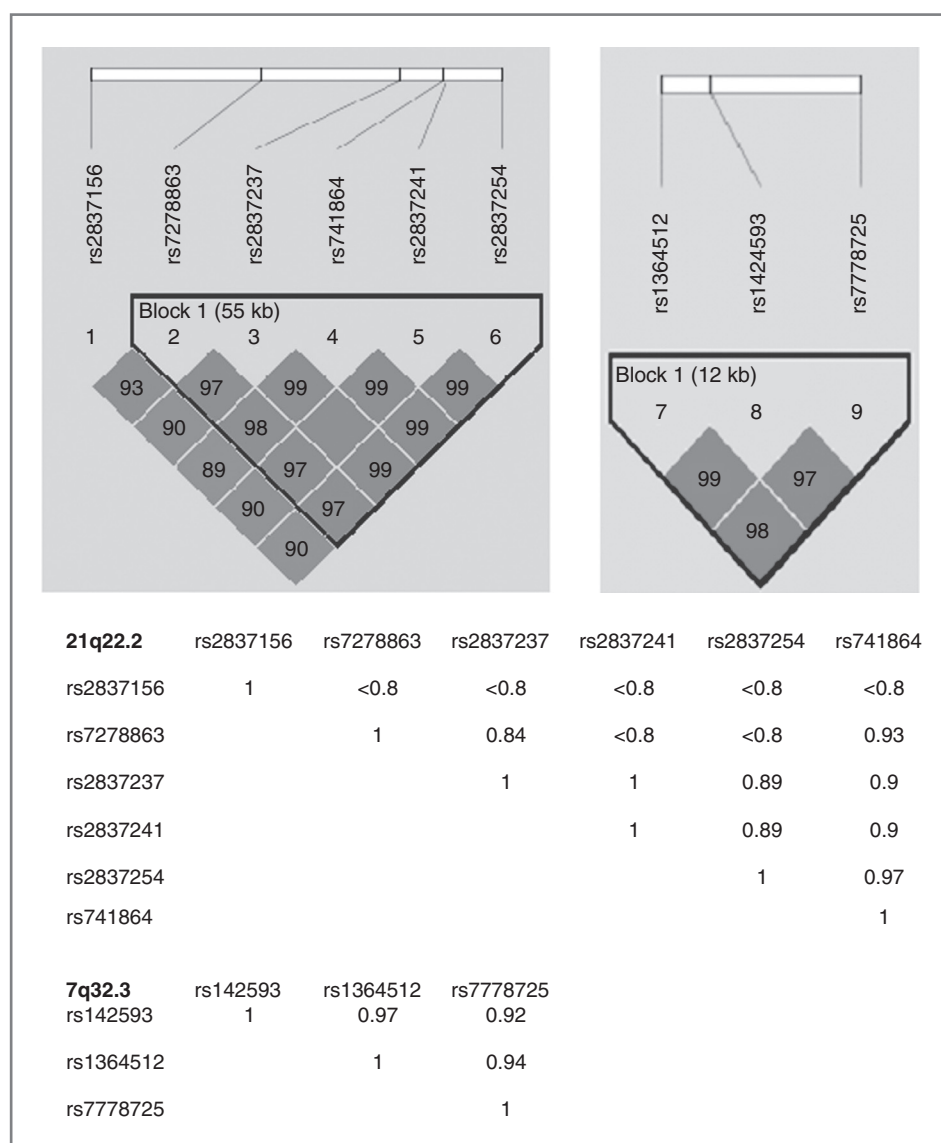


Figure 2. LD analysis of SNPs on 21q22.2 and 7q32.3 by pairwise r^2 . The 6 IGSF-5 related SNPs are within very tight linkage disequilibrium (LD) region.

For the additional susceptibility evaluation cohorts, samples were genotyped on Illumina Infinium SNP arrays, ranging from the Hap300 (for VQ58) to the Hap1M (for Australia). Details about genotyping and quality control for these studies have been provided previously (5). Ethics Committees approved these five studies and samples were collected in accordance with the tenets of the Declaration of Helsinki.

Among the top 19 SNPs identified from the APC trial, 12 SNPs had genotype data available from the CORGI, Scotland, VQ58, or Australia GWAS. Nine of these SNPs were typed in all four studies (rs1381392, rs17651822, rs17781398, rs16909065, rs9582985, rs2837156, rs2837241, and rs741864) and three were typed in three (rs13085889, rs1424593, and rs2837237; Supplementary Table S1).

Statistical analysis

To assess the strength of association between genotype and advanced-adenoma risk, a per allele unconditional logistic regression model was used to estimate ORs and their corresponding 95% confidence intervals (CI). For the APC trial, genotype–phenotype interactions were evaluated for sex, age at trial entry (\leq age 60 years vs. $>$ age 60 years), and family history (first-degree relatives with colorectal cancer). Genotype–environment interactions were evaluated for aspirin use at baseline and treatment with celecoxib. The Breslow–Day test was used to test the homogeneity of ORs. PLINK (<http://pngu.mgh.harvard.edu/~purcell/plink/>), STATA, and SAS were used to conduct all the

analysis. Genome-wide significance was defined as false discovery rate less than 0.05, which corresponds to an unadjusted $P = 7.4 \times 10^{-7}$ in this analysis (8, 9).

Results

Of note, 1,406 evaluable Caucasian patients were genotyped from the APC trial (4). Eight SNPs were identified by association with on-study development of advanced adenomas at a genome-wide level of significance: rs2837156, rs7278863, rs2837237, rs2837241, rs2837254, rs741864, all at 21q22.2, and rs1381392 and rs17651822, both at 3p24.1 (Table 1). The associations between the six SNPs in the 21q22.2 region and advanced adenoma development were all highly significant (unadjusted $P = 10^{-8}$ – 10^{-9}) with ORs per allele ranging from 2.22 to 2.55. All six SNPs in the 21q22.2 region were located near the coding region for the adherens junction protein, *IGSF5*, and five of these SNPs (rs7278863, rs2837237, rs741864, rs741864, and rs2837241) were in strong linkage disequilibrium ($r^2 = 0.8$ – 1 ; Fig. 1). For the 3p24.1 signal, the OR for genotype rs1381392 was 2.01 (95% CI, 1.52–2.65; unadjusted $P = 7.4 \times 10^{-07}$), and that for rs17651822 was 2.16 (95% CI, 1.61–2.91; unadjusted $P = 2.1 \times 10^{-7}$).

Eleven SNPs (rs11886781 at 2p24.2, rs13085889 at 3q22.2, rs1424593, rs1364512, and rs7778725 at 7q32.3, rs16909065 and rs16909036 at 9q33.2, rs17654765, rs1535989, and rs9582985 at 13q33.2) were associated with moderate (\sim 2-fold) ORs for advanced adenoma detection, but the associations did not reach genome-wide significance ($P \leq 10^{-6}$). Of these 11 SNPs,

Table 1. APC trial advanced adenoma susceptibility loci^a

Chromosome region	SNP	Position (bp)	Alleles	MAF	P	OR	Gene
2p24.2	rs11886781	18154780	A C	0.08	9.7E-06	2.25 (1.56–3.25)	KCNS3
3q22.2	rs13085889	135843760	A C	0.29	8.8E-06	1.77 (1.37–2.29)	EPHB1, KY
3p24.1	rs1381392	28724318	A G	0.18	7.4E-07	2.01 (1.52–2.65)	
3p24.1	rs17651822	28695130	A G	0.14	2.1E-07	2.16 (1.61–2.91)	
7p14.3	rs17781398	30807966	A G	0.10	9.0E-06	0.19 (0.08–0.43)	FAM188b
7q32.3	rs1424593	131605541	C A	0.50	9.1E-06	0.56 (0.44–0.73)	PLXNA4
7q32.3	rs1364512	131602384	C A	0.49	8.6E-06	0.56 (0.42–0.71)	PLXNA4
7q32.3	rs7778725	131614936	G A	0.49	4.0E-06	0.55 (0.42–0.71)	PLXNA4
9q33.2	rs16909065	121597606	A G	0.05	3.6E-06	2.59 (1.71–3.93)	
9q33.2	rs16909036	121587049	G A	0.05	3.7E-06	2.59 (1.71–3.93)	
13q33.2	rs1535989	104820723	G A	0.11	8.9E-06	2.09 (1.50–2.91)	
13q33.2	rs17654765	104828038	A G	0.10	4.7E-06	2.14 (1.53–2.98)	
13q33.2	rs9582985	104829133	C A	0.11	9.3E-06	2.05 (1.48–2.83)	
21q22.2	rs2837156	40048557	G A	0.12	3.2E-07	2.22 (1.62–3.03)	IGSF5
21q22.2	rs7278863	40087578	A G	0.10	1.4E-08	2.48 (1.80–3.42)	IGSF5
21q22.2	rs2837237	40119727	G A	0.12	3.6E-09	2.48 (1.82–3.38)	
21q22.2	rs2837241	40130476	A C	0.12	3.7E-09	2.48 (1.82–3.38)	
21q22.2	rs2837254	40143171	A G	0.11	2.9E-09	2.55 (1.86–3.51)	
21q22.2	rs741864	40129665	A G	0.11	1.1E-08	2.48 (1.80–3.41)	

^aThe total number of subjects is 1,406, of which 139 developed advanced adenomas.

Table 2. Meta-analysis using adenoma or colorectal cancer as a composite outcome

SNP	N	P	P(R)	OR	OR(R)	Q	I
rs13085889	4	0.1048	0.1048	1.0575	1.0575	0.5438	0
rs1381392	4	0.4636	0.4636	1.0295	1.0295	0.4186	0
rs1424593	3	0.405	0.405	1.027	1.027	0.4233	0
rs1535989	4	0.012	0.012	1.1304	1.1304	0.7925	0
rs16909065	4	0.1273	0.1273	0.9054	0.9054	0.498	0
rs17651822	4	0.689	0.689	1.0176	1.0176	0.8499	0
rs17781398	4	0.7972	0.7972	1.016	1.016	0.9487	0
rs2837156	4	0.8017	0.8017	0.9881	0.9881	0.4326	0
rs2837210	4	0.8706	0.9766	1.0083	0.9983	0.3114	16.05
rs2837237	3	0.3464	0.3464	0.9379	0.9379	0.413	0
rs2837241	4	0.938	0.8585	0.9963	0.9907	0.3395	10.69
rs741864	4	0.6248	0.6248	0.971	0.971	0.6068	0
rs9582985	4	0.05468	0.05468	1.1188	1.1188	0.9416	0

six mapped to gene coding regions: rs11886781 to *KCNS3*, rs17781398 to *FAM188b*, rs13085889 to *EPHB1* and *KY*, and rs1424593, rs1364512, and rs7778725 all to *PLXNA4* (Table 1 and Fig. 1).

There are no comparable adenoma chemoprevention cohorts currently available for validation of the APC GWAS results. We therefore further examined APC trial results using GWAS data from four nonoverlapping colorectal cancer case-control series of European ancestry, one of which (CORGI) also included advanced adenoma cases (5). Among the 19 advanced-adenoma risk SNPs with a nominal significance level of $P \leq 10^{-6}$, 12 were genotyped in at least three of the available four colorectal cancer GWA studies (Supplementary Table S1). Allelic frequencies of each variant and the corresponding associations with colorectal cancer phenotype were accessed in each of the four case-control samples. The results of the meta-analysis for overall associations with colorectal cancer risk are reported in Table 2 and Supplementary Fig. S3.

One of the 19 SNPs identified in the APC trial, rs1535989, was replicated in the independent colorectal cancer cohorts, with an OR for colorectal cancer development of 1.12 (95% CI, 1.019–1.23; $P = 0.019$). There was no evidence of inter-study heterogeneity ($P_{\text{het}} = 0.71$; $I^2 = 0.0\%$). An additional exploratory meta-analysis was performed, combining all five studies and using either advanced adenoma or colorectal cancer as the outcome (Table 2). SNP rs9582985 originally identified in the APC cohort showed marginally significant association with outcome (OR, 1.11; $P = 0.055$).

Clinical data from the APC trial were used to further characterize rs1535989 by examining the association of this variant with other susceptibility factors for advanced colorectal neoplasia, including age, sex, aspirin use at baseline, family history of colorectal cancer, and on-study treatment with celecoxib. SNP and environmental factors interaction terms were included in the model. SNP rs1535989 showed statistically significant interactions with subjects' age ($P = 0.0016$), sex ($P = 0.0057$), and aspirin use at baseline

($P = 0.02$). The associations with advanced neoplasia were stronger in older individuals (>60; OR, 3.20; 95% CI, 2.10–4.87), males (OR, 2.74; 95% CI, 1.89–3.97), and those using aspirin at baseline (OR, 3.63; 95% CI, 2.06–6.40; Table 3). There were no statistically significant interactions with colorectal cancer family history or on-study treatment with celecoxib.

Discussion

Among the approximately 145,000 colorectal cancer cases diagnosed per year in the United States, only 5% represent autosomal dominant predisposition syndromes, with the majority of these involving either hereditary non-polyposis colon cancer (HNPCC) or familial adenomatous polyposis (FAP). An additional 20% to 25% of colorectal cancer cases show a familial association without precise genetic characterization, and the majority of colorectal cancers occur in individuals without a family history of the disease. Self-reported family history does not accurately

Table 3. Genotype-phenotype/environment interactions for SNP rs1535989

Phenotype	OR	Interaction (P)
Age	≤60	0.91 (0.42–1.76)
	>60	3.20 (2.10–4.87)
Sex	Female	0.65 (0.25–1.68)
	Male	2.74 (1.89–3.97)
Family history	No	2.23 (1.51–3.28)
	Yes	1.74 (0.88–3.43)
Prior aspirin use	No	1.58 (1.03–2.42)
	Yes	3.63 (2.06–6.40)
Treatment	Placebo	1.63 (1.03–2.58)
	200 mg	2.51 (1.27–4.95)
	400 mg	2.43 (1.16–5.07)
		NS

assess the inherited risk of advanced adenomas because patients' knowledge of their family history of colorectal adenomas is often unknown or incomplete (10). Recent GWAS from members of this collaboration have identified 18 colorectal cancer susceptibility variants with minor allele frequencies ranging from 0.07 to 0.48 that each convey a small degree of risk modification (OR per allele, 0.87–1.35; refs. 11–15). The results presented here expand these data to address inherited susceptibility for developing advanced adenomas that represent targets for colorectal cancer prevention. In addition to the studies whose data were used here, there have been a number of other GWAS with colorectal cancer or colorectal adenomas as the primary phenotype (16–20). These have yielded a substantial number of possible susceptibility variants, most conveying modestly altered risk. A recent case-control meta-analysis from 14 studies identified SNPs on 2q32.3 (rs11903757), 1q25.3 (rs10911251), 12p13.32 (rs3217810), and 12q24.21 (rs59336) that represented ORs ranging from 0.84 to 1.15 (16).

The APC trial was designed to determine whether the selective cyclooxygenase-2 inhibitor, celecoxib, prevented adenomas in patients at high risk for colorectal cancer. Eligibility criteria required that participants have at least one prior adenoma >6 mm in size, or multiple adenomas. During the 5 years of endoscopic surveillance, 21.3% of APC trial participants randomized to placebo developed recurrent advanced adenomas (21). This rate was decreased to 12.5% in patients receiving celecoxib 200 mg twice daily ($P < 0.0001$), however, concerns over cardiovascular toxicity currently prohibit the use of celecoxib for routine colorectal cancer chemoprevention (22). Results presented here showed that advanced adenomas were twice as likely to occur in APC trial participants with variant rs1535989, and that this increased risk was not affected by celecoxib treatment. For males or older individuals, the risk was more than 3-fold higher than that for females or participants younger than 60. The observed interaction between baseline aspirin use and advanced-adenoma risk is particularly interesting. Aspirin use reduces the incidence of colorectal adenomas and colorectal cancer, and subjects enrolled in the APC trial who used aspirin at baseline were those who developed adenomas despite aspirin use. These individuals may therefore have constituted a higher risk subset because they were relatively resistant to aspirin chemoprevention. The analyses conducted here showed that APC trial participants who both developed adenomas while taking aspirin and had variant rs1535989 demonstrated a 3.63-fold increase in advanced-adenoma risk during surveillance. If this association can be confirmed in other studies, and other variants of similar effect are found, then genotyping will represent a useful method to target high-risk patients for preventive treatments including more frequent colonoscopic screening.

Additional results from this GWAS suggest areas for further research about the molecular basis of colorectal neoplasia. Variants at rs2837156, rs7278863, rs2837237, rs2837241, rs2837254, and rs741864 are in close associa-

tion at 21q22.2 (Fig. 2). Two of these SNPs, rs2837156 and rs7278863, are within the coding region of *IGSF5*, a gene encoding a transmembrane protein whose murine homolog, JAM4, binds to the tumor suppressor MAGI-1 at intestinal epithelial tight junctions (23). To explore the potential effect of *IGSF5* on the prognosis of patients with colorectal cancer, microarray expression data from the GEO data set (GSE14333) were retrieved for 229 Dukes A, B, and C patients (24). The gene expression profile was performed with Affymetrix u133p2 platform. Our preliminary analysis of these data indicated that the overexpression of *IGSF5* is associated with significantly worse relapse-free survival (unadjusted $P = 0.000004$; bonferroni adjusted $P = 0.00085$; Supplementary Fig. S4). In addition, rs1424593, rs1364512, and rs7778725 all involve *PLXNA4*, a member of the plexin family located on chromosome 7. Plexins are transmembrane, secreted, and GPI-anchored semaphorins that modulate the adhesive and migratory properties of malignant cells. The protein product of *PLXNA4* forms stable complexes with FGFR1 and VEGFR-2 tyrosine kinase receptors and enhances both VEGF-induced VEGFR-2 phosphorylation and β FGF-induced cell proliferation (25). Finally, *EPHB1* encodes a ligand that binds to an Eph receptor tyrosine kinase to mediate bidirectional signaling required for intestinal epithelial homeostasis (26). EphB-ephrin B interactions regulate cell adhesion, migration, and positioning, and play an important role in colorectal tumor progression (27).

The limitation of our current study resides in the following two aspects. The limited number of advanced adenoma cases in the APC trial restricted the power to identify more advanced adenoma susceptibility SNPs. In addition, the colorectal cancer/adenoma cases with a strong family history of colorectal cancer were not excluded from replication datasets. This might under/overestimate the association between identified SNPs and sporadic colorectal-cancer risks.

In summary, this study identified 19 SNPs associated with advanced-adenoma risk at a level of $P \leq 10^{-6}$. Of these, 12 SNPs were tested in a meta-analysis using independent datasets to evaluate their association with colorectal cancer development, and rs1535989 was also associated with increased risk of both advanced adenomas and colorectal cancer. In addition, eight of the variants identified in the APC trial mapped to coding regions of genes previously implicated in colorectal cancer progression, and warrant further study to confirm their role in modifying tissue-specific biologic function.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: J. Wang, L.G. Carvajal-Carmona, A.G. Zauber, APC Trial Collaborators, M. Kubo, M.J. Ratain, I. Tomlinson, M.M. Bertagnolli
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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): L.G. Carvajal-Carmona, APC Trial Collaborators, M. Kubo, K. Matsuda, M. Dunlop, R.S. Houlston, O. Sieber, L. Lipton, P. Gibbs,

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): J. Wang, L.G. Carvajal-Carmona, J.-H. Chu, A.G. Zaubler, APC Trial Collaborators, M. Dunlop

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