Research Article

Cancer Epidemiology, Biomarkers & Prevention

Germline Genetic Variants in the Wnt/β-Catenin Pathway as Predictors of Colorectal Cancer Risk

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Abstract

Background: The Wnt/β-catenin signaling pathway plays a key role in stem cell maintenance in the colorectum. Rare high-penetrance genetic mutations in components of this pathway result in familial colorectal cancer, yet the impact of common, germline variants remains unknown.

Methods: We assessed 172 variants in 26 genes from the Wnt/β-catenin pathway in 809 colorectal cancer cases and 814 healthy controls, followed by replication of the top findings in another 691 cases and 775 controls. *In silico* informatic tools were used to predict functional effects of variants.

Results: Eighteen SNPs in the pathway were significantly associated with colorectal cancer risk ($P < 0.05$) in the discovery phase. We observed a significant dose-response increase in colorectal cancer risk by number of risk genotypes carried ($P = 4.19 \times 10^{-8}$). Gene-based analysis implicated *CSNK1D* ($P = 0.014$), *FZD3* ($P = 0.023$), and *APC* ($P = 0.027$) as significant for colorectal cancer risk. In the replication phase, *FZD3*:rs11775139 remained significantly associated with reduced risk with a pooled OR of 0.85 [95% confidence interval (CI), 0.76–0.94, $P = 0.001$]. Although borderline significant in the replication population, *APC*:rs2545162 was highly significant in the pooled analysis—OR, 1.42; 95% CI, 1.16–1.74; $P = 0.00085$. Functional assessment identified several potential biologic mechanisms underlying these associations.

Conclusions: Our findings suggest that common germline variants in the Wnt/β-catenin pathway may be involved in colorectal cancer development.

Impact: These variants may be informative in colorectal cancer risk assessment to identify individuals at increased risk who would be candidates for screening. *Cancer Epidemiol Biomarkers Prev; 25(3); 540–6. ©2016 AACR.*

Introduction

Colorectal cancer is the third leading cancer in the United States in which approximately 93,000 newly diagnosed cases and 50,000 estimated deaths are expected to occur in 2015 (1). Through the combination of established screening protocols and colonoscopic polypectomy, colorectal cancer is a curable disease when detected at an early stage (2). However, the 5-year survival rate for colorectal cancer has remained unchanged at approximately 64% (3). This is due, in part, to the current screening recommendations in the general population being based primarily on age. There is a need for approaches to better identify those at risk and refine screening recommendations based on a more comprehensive assessment of colorectal cancer risk.

An increased risk of colorectal cancer is associated with certain environmental risk factors, including diet (i.e., high fat and red meat), obesity, a sedentary lifestyle, smoking, alcohol consumption, and chronic inflammatory disease (i.e., Crohn's disease) (4–8). Risk assessment studies have demonstrated that environmental factors alone have only a modest effect on colorectal cancer susceptibility (8). It has been well established that genetic alterations also play an important role in the etiology of colorectal cancer (9–11). Rare, high-penetrance genetic mutations in mismatch repair genes, *MLH1*, *MSH2*, *PMS1*, and *PMS2*, account for approximately 1% to 3% of colorectal cancer cases (12). Genetic mutations in *APC* and *MYUTH* have also been reported in colorectal cancer cases (13). Solidifying the link between inherited genetic factors and cancer risk, a twin study reported that 35% of colorectal cancer risk may be due to heritable factors (14). As a step to identify the genetic factors influencing colorectal cancer risk, a series of genome-wide association studies have identified several common, germline colorectal cancer susceptibility loci (15–19). Although common in the population, the predictive power for risk of colorectal cancer was low for individual genetic variants identified by GWAS. For example, the loci at 8q23.3 and 10p14, only account for 10% and 4% of the familial risk, respectively (16, 20). This indicates that there are other undiscovered genetic variants that impact colorectal cancer risk.

The Wnt/β-catenin signaling pathway is responsible for maintaining stem cell homeostasis. A low number of stem cells give rise to colon epithelial cells (colonocytes) that line the bottom of the crypts (compartments below the luminal layer). There is strong evidence linking genetic mutations in components of the Wnt/β-catenin pathway to the development of colorectal cancer. First and foremost, genetic mutations in adenomatous polyposis coli (*APC*) were discovered as a heritable predisposition for colorectal cancer in individuals with familial adenomatous polyposis (FAP; ref. 9). As a tumor suppressor, the loss or inactivation of *APC* initiates tumorigenesis and helps drive progression of colorectal cancer (21). Inactivation of APC prevents degradation

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doi: 10.1158/1055-9965.EPI-15-0834

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of β-catenin and contributes to β-catenin nuclear translocation. In the absence of the Wnt ligand binding to its receptors low-density lipoprotein receptor–related proteins 5 and 6 (LRP5/6) and the seven-transmembrane receptor Frizzled, β-catenin is recruited to its "destruction complex" containing Axin1 and APC, which then allows phosphorylation by casein kinase 1 (CK1) and glycogen synthase kinase-3 beta (GSK3B), and subsequent degradation of β-catenin through ubiquitinylation and proteasome complex. This process prevents the translocation of β-catenin to the nucleus and maintains repression of β-catenin target genes by the T-cell factor/lymphoid enhancer factor (TCF/LEF) (22, 23). Upon binding of Wnt, the Frizzled and LRP5/6 complex recruits the scaffolding protein, Dishevelled (Dvl), to the cell membrane, preventing the phosphorylation and degradation of β-catenin. β-Catenin accumulates in the nucleus where it activates transcription of several target genes implicated in cell proliferation, differentiation, and motility (24). Defects in the Wnt/β-catenin pathway result in uncontrolled cell proliferation, including the stem cells lining the colon.

However, no studies have investigated the role of common, germline genetic variants in this key signaling pathway on susceptibility to colorectal cancer. Identification of novel genetic biomarkers associated with susceptibility to colorectal cancer is critical for improved screening guidelines and early detection that can contribute to reducing colorectal cancer-associated mortality. We hypothesized that common genetic variations within the Wnt/β-catenin signaling pathway are associated with increased colorectal cancer risk, and tested this hypothesis in a large, two-stage case–control study that included 1,502 colorectal cases and 1,589 healthy controls.

Materials and Methods

Patient population

Colorectal cancer cases included in this study were recruited from MD Anderson Cancer Center and histologically confirmed. There were no age, gender, ethnicity, and cancer stage restrictions for recruitment. Control subjects were derived from an existing pool of participants from an ongoing molecular epidemiologic case–control study at MD Anderson (25). This healthy population included people from all racial, ethnic, and socioeconomic groups that make up the Houston population. With the exclusion of non-melanoma skin cancer, controls had no prior history of cancer and were matched to cases by age (±5 years) and gender. Demographics and epidemiology data were collected for all participants. For this analysis, we restricted our population to only non-Hispanic whites to minimize potential confounding effects from population stratification. A randomly selected set of 811 cases and 814 controls were included in the discovery phase with an additional set of 691 cases and 775 controls included as a replication phase. All study participants provided peripheral blood samples for research purposes. Genomic DNA was isolated from these samples using the QIAamp DNA Kit and banked for future use. The study has been approved by the Institutional Review Board of MD Anderson Cancer Center and written informed consent was obtained from all study participants.

Discovery genotyping

A custom Illumina BeadArray genotyping chip was designed to assess genetic variation within the Wnt/β-catenin signaling pathway. Pathway genes were identified using BioCarta, KEGG, and literature review. A tagging SNP approach was used to identify common variants within these genes, using Tagger (26) based on a region containing 10 kb flanking regions upstream and downstream of each gene with r^2 of 0.8 or higher and a minor allele frequency (MAF) of >5% in the CEU population of HapMap (release 27, phase II+III). A total of 172 genetic variants from 26 genes, with an emphasis on core components of the canonical Wnt/β-catenin signaling pathway, were selected for inclusion. Genotyping was performed in the discovery population (811 cases and 814 controls) on Illumina's BeadArray platform according to the manufacturer's instructions with inclusion of replicates, internal quality controls, and negative controls to ensure genotyping accuracy. Genotypes were auto-called using the BeadStudio software (Illumina).

Replication genotyping

The top eight variants identified in the discovery analysis were selected for further genotyping in the replication population (691 cases and 775 controls). Genotyping was performed using TaqMan Genotyping Assays using the 384-well ABI 7900HT Sequence Detection System following the standard protocol with inclusion of appropriate quality control, negative controls, and duplicates.

Statistical analysis

The χ^2 test was used to evaluate each SNP for deviation from the Hardy–Weinberg equilibrium (HWE) in the controls with SNPs with P values <0.01 removed from further analysis. Univariate and multivariate logistic regression was performed in both the discovery and replication phases to estimate odds ratios (ORs) and 95% confidence intervals (CI) for each variant while adjusting for age and gender. Analysis included assessment of all three genetic models of inheritance (dominant, recessive, and additive), with the model reaching the highest degree of statistical significance reported. The dominant model was the sole model assessed for SNPs with homozygous variant genotypes of <5% in the cases or controls. Bootstrap resampling for 500 iterations was conducted for the findings in the discovery population with the number of results reaching $P < 0.05$ in that sampling recorded. Cumulative analysis was used to evaluate the combined effect of the top eight variants identified in the discovery phase on colorectal cancer risk. We performed a gene-based analysis using VEGAS (Versatile Gene-based Association Study) with an empirical P value for each gene calculated on the basis of genotypes of the entire discovery population dataset (27).

In silico functional assessment

HaploReg v3 (28), an online database that annotates variants from 1000 Genomes Pilot 1 SNP dataset ($r^2 = 0.8$, CEU population), was used to identify proxy variants in high LD with genotyped variants in the top 3 candidate genes (overall $P < 0.05$; *APC*, *FZD3*, and *CSNK1D*) that were identified by gene-based analysis. The genotyped variants that were queried included: *APC*:rs2545162, rs9326862, rs2431507, rs2439595, rs2546108, rs2707761, rs459552, *FZD3*:rs11775139, rs164658, rs17438384, rs1908916, rs352199, rs352212, rs3922392, rs6558063, and *CSNK1D*:rs11653735, rs7209167, rs7503429, and rs9901910. A total of 264 proxy SNPs were identified for *APC*, 169 proxy SNPs for *FZD3*, and 3 proxy SNPs for *CSNK1D*.

Annotated functional effects were assessed in HaploReg v3, as well as SNiPA (SNP annotator; http://www.snipa.org/; ref. 29), a variant-centered genome browser and interactive tool that contains annotations from phase III version 5 of the 1000 Genomes Project. The eQTL effects for each variant were examined using an eQTL browser (http://www.genenetwork.nl/bloodeqtlbrowser; ref. 30).

Results

Characteristics of the study population

The characteristics of the 811 colorectal cancer cases and 814 controls comprising the discovery population and 691 colorectal cancer cases and 775 controls for the replication population are presented in Table 1. There were no differences in the distributions of age and gender between cases and controls in either discovery or replication sets. The discovery population was slightly older than the replication population and had a slightly larger portion of male participants.

Risk associated with individual Wnt/β-catenin pathway SNPs

A total of 172 SNPs within 26 genes of the Wnt/β-catenin pathway were analyzed in 811 cases and 814 controls with one SNP removed due to deviation from HWE. We identified 18 SNPs with *P* values <0.05 that were significantly associated with colorectal cancer risk as presented in Table 2. The effects of these SNPs were consistent in unadjusted and adjusted analyses. The top two variants *FZD3*:rs17438384 and *APC*:rs2545162 each conferred a greater than 1.6-fold increase in risk with ORs of 1.63 (95% CI, 1.19–2.24; $P = 0.0024$) and 1.65 (95% CI, 1.23–2.22; $P = 0.00090$), respectively, under the recessive model. The findings for these two variants were supported by bootstrap analysis with each remaining significant in 100% of samples. An additional six SNPs were also shown to be significant ($P < 0.05$) with over 80% of the bootstrap resamplings significant, including *APC*:rs459552, *GSK3B*:rs3732360, *CTNNB1*:rs4135385, *LRP5*:rs624947, *LRP5*:rs638051, and *FZD3*:rs11775139. These eight variants were selected for further analysis and genotyping in the replication population.

Cumulative effect of Wnt/β-catenin pathway genetic variants

To better define an individual's risk of colorectal cancer, the cumulative effect of the top eight SNPs was assessed. A clear dose–response relationship was evident with an increase in risk for each increase in the number of risk genotypes (P_{trend} 4.19×10^{-8}; Table 3). Compared with individuals with only one risk genotype, those with three such genotypes had a 1.86-fold increase in risk (95% CI, 1.30–2.65; $P = 6.7 \times 10^{-4}$), which increased to 2.19-fold for individuals carrying four to seven of these genotypes (95% CI, 1.49–3.21; $P = 6.0 \times 10^{-5}$). No one in the study population carried all eight of the risk genotypes.

Gene-based analysis of Wnt/β-catenin variation

Our pathway-based study design identified a panel of tagging SNPs across each of the candidate genes. Therefore, we conducted a gene-based analysis to summarize the effect of individual variants within the context of a gene using the entire discovery dataset of 172 variants. VEGAS identified three candidate genes, *CSNK1D* ($P = 0.014$), *FZD3* ($P = 0.023$), and *APC* ($P = 0.027$), that were significantly associated with colorectal cancer risk. Interestingly, none of the individual variants genotyped in *CSNK1D* were highly significant in the discovery main effects analysis.

Replicated SNPs in the Wnt/β-catenin pathway associated with colorectal cancer risk

To replicate our findings, we genotyped the top eight variants that had bootstrap generated *P* values <0.05 for over 80% of the iterations in the discovery analysis in a population of 691 colorectal cancer cases and 775 controls (Table 4). *FZD3*:rs11775139 (OR, 0.80; 95% CI, 0.69–0.93; $P = 0.0034$) was significant in this replication population with a similar magnitude of effect as the discovery population. In the pooled population, the finding for *FZD3*:rs11775139 increased in significance ($P = 0.0011$) with a resulting 15% reduction in risk for those carrying the two variant alleles. Another variant, *APC*:rs2545162, was borderline significant in the replication population (OR, 1.15; 95% CI, 1.00–1.34; $P = 0.056$), but reached significance in the pooled population (OR, 1.42; 95% CI, 1.16–1.74; $P = 0.00085$). *GSK3B*:rs3732360 (OR, 0.78; 95% CI, 0.78; $P = 0.088$), *FZD3*:rs17438384 (OR, 1.21; 95% CI, 0.96–1.53; $P = 0.099$), *LRP5*:rs638051 (OR, 1.76; 95% CI, 0.97–1.43; $P = 0.10$), *LRP5*:rs624947 (OR, 1.09; 95% CI, 0.94–1.26; $P = 0.24$), and *CTNNB1*:rs4135385 (OR, 1.11; 95% CI, 0.96–1.28; $P = 0.15$) were not significant in the replication or pooled populations. *APC*:rs459552 did not pass quality control measures in the replication genotyping and therefore, was excluded (NA) from the replication and pooled results in Table 4.

In silico functional prediction of Wnt/β-catenin pathway variants

The tagging SNPs directly genotyped in this study may not be the causal variants, but serve as proxies for the true causal variants due to linkage disequilibrium relationships. Therefore, we conducted *in silico* predictions to identify variants located in putative functional elements that represent potential mechanisms for the observed genetic associations (Table 5). Although

Table 1. Host characteristics

	Discovery		Replication	
	Case *N* (%)	Control *N* (%)	Case *N* (%)	Control *N* (%)
Total	811	814	691	775
Age				
Mean (SD)	60.63 (10.4)	61.08 (10.4)	54.06 (12.3)	57.02 (12.3)
Gender				
Male	551 (67.9)	557 (68.4)	353 (51.1)	396 (51.1)
Female	260 (32.1)	257 (31.6)	338 (48.9)	379 (48.9)

Table 2. SNPs in the Wnt/β-catenin pathway associated with colorectal cancer risk

SNP	Gene	Case genotypes	Control genotypes	MAF	MOI	OR (95% CI)	*P*	OR[a] (95% CI)	*P*	Bootstrap[b]
rs17438384	*FZD3*	356\347\108	384\358\71	0.33	rec	1.61 (1.17–2.20)	0.0034	1.63 (1.19–2.24)	0.0024	**500**
rs2545162	*APC*	340\340\128	324\401\84	0.36	rec	1.62 (1.21–2.18)	0.0012	1.65 (1.23–2.22)	0.00090	**500**
rs459552	*APC*	467\288\54	473\305\32	0.24	rec	1.74 (1.11–2.72)	0.016	1.76 (1.12–2.76)	0.014	**495**
rs3732360	*GSK3B*	420\337\49	415\324\75	0.28	rec	0.64 (0.44–0.93)	0.018	0.64 (0.44–0.93)	0.018	**485**
rs4135385	*CTNNB1*	460\298\52	503\263\45	0.23	dom	1.24 (1.02–1.52)	0.032	1.25 (1.03–1.53)	0.026	**472**
rs624947	*LRP5*	366\339\105	394\343\76	0.32	rec	1.44 (1.06–1.97)	0.021	1.43 (1.05–1.96)	0.025	**457**
rs638051	*LRP5*	280\386\143	307\396\110	0.40	rec	1.37 (1.05–1.80)	0.022	1.35 (1.03–1.77)	0.029	**429**
rs11775139	*FZD3*	266\416\129	254\397\163	0.43	rec	0.76 (0.59–0.97)	0.031	0.75 (0.58–0.97)	0.030	**421**
rs17653687	*TCF7*	537\252\19	580\206\27	0.17	dom	1.26 (1.02–1.55)	0.034	1.25 (1.02–1.55)	0.036	32
rs3867143	*LRP5*	559\234\18	598\193\23	0.16	dom	1.25 (1.01–1.55)	0.044	1.25 (1.01–1.55)	0.040	338
rs4760662	*WNT1*	319\365\124	282\403\128	0.39	dom	0.81 (0.67–1.00)	0.046	0.81 (0.66–0.99)	0.041	338
rs5757037	*CSNK1E*	302\405\101	308\375\131	0.38	rec	0.74 (0.56–0.99)	0.039	0.75 (0.57–0.99)	0.044	314
rs352199	*FZD3*	332\384\94	317\375\121	0.37	rec	0.75 (0.56–1.00)	0.052	0.75 (0.56–1.00)	0.046	271
rs11054704	*LRP6*	610\181\17	576\219\16	0.14	dom	0.80 (0.64–0.99)	0.042	0.80 (0.64–0.99)	0.049	238
rs199494	*WNT3*	255\391\161	288\391\135	0.42	add	1.16 (1.01–1.33)	0.041	1.16 (1.01–1.33)	0.039	91
rs164658	*FZD3*	408\339\64	370\360\83	0.31	add	0.84 (0.73–0.98)	0.027	0.84 (0.72–0.97)	0.022	55
rs7503429	*CSNK1D*	235\419\156	282\398\133	0.43	add	1.20 (1.04–1.38)	0.013	1.20 (1.04–1.38)	0.013	19
rs11653735	*CSNK1D*	557\224\26	526\246\38	0.18	add	0.84 (0.70–1.00)	0.046	0.84 (0.70–1.00)	0.047	1

Abbreviations: add, additive; dom, dominant; MOI, model of inheritance; rec, recessive.

[a]Adjusted for age and gender.

[b]Number of resamplings out of 500 that remained significant ($P < 0.05$), including those selected for replication in bold font.

FZD3:rs11775139, an intronic variant, was predicted to be a weak transcriptional regulator via its location in an enhancer-like region, several proxy SNPs in high LD with *FZD3*:11775139, *FZD3*:rs6997072, *FZD3*:rs6558067, *FZD3*:rs11781193, *FZD3*:rs2323018, and *FZD3*:rs2874941, included those predicted to have strong transcriptional regulatory effects based on their locations in promoters, enhancers, and upstream and downstream transcriptional start sites. Genotyped variants, *FZD3*:rs164658 located in the 3′ flanking region and *FZD3*:rs1908916 located in the 5′ flanking region, were predicted to have functional effects via their location in enhancer regions and promoter sites, respectively. One of the *FZD3* variants (rs164658) was identified as a direct eQTL regulating expression of *FZD3*.

We found that *APC*:rs2545162, an intronic variant, was predicted to strongly affect transcription via its location in an enhancer-like region of *APC*. Several other potential causal variants include, *APC*:rs2545164, *APC*:rs563556, *APC*:rs1734243, and *APC*:rs2431238. These intronic variants were also predicted to affect transcriptional regulation via their location in enhancer-like regions. Interestingly, we found three variants in *APC* (rs2545162, rs563556, and rs459552) to have *cis*-eQTL effects on *REEP5* (receptor accessory protein 5), located approximately 30 kb downstream from *APC*.

Our gene-based analysis also implicated *CSNK1D* as important for colorectal cancer risk. Our study directly genotyped four SNPs in *CSNK1D*: rs11653735, rs7209167, rs9901910, and rs7503429. The query for proxy SNPs identified three additional variants in high LD with *CSNK1D*:rs7503429: rs66871014, rs35121878, and rs12601778. No proxy SNPs were found in high LD with *CSNK1D*:rs11653735, *CSNK1D*:rs7209167, and *CSNK1D*:rs9901910. Functional prediction was assessed for this set of seven variants. *CSNK1D*:rs11653735, significant in the discovery main effect analysis, is an intronic variant located within a nonsense mediated decay region of *CSNK1D*. It is predicted to have direct regulatory effects on genes through *cis*-eQTL relationships with three genes: *CSNK1D*, *CD7*, and *STRA13*. *CSNK1D*:rs11653735 is located on chromosome 17 approximately 64 kb upstream to *CD7* and 227 kb downstream to *STRA13*. *CSNK1D*:rs7209167, located within an intronic region, was predicted to have weak and active effects in promoter regions and regulatory effects on upstream and downstream TSS. *CSNK1D*:rs9901910, located within an intronic region of *CSNK1D* approximately 202 kb downstream to its *cis*-eQTL gene *DCXR*, is predicted to have strong regulatory effects in enhancer regions. A significant variant from the discovery main effect analysis, rs7503429, was predicted to have weak effects on promoters and strong effects on enhancers and is located in *SLC16A3*, which is 5′ of *CSNK1D*. As one of its 3 proxy SNPs, rs66871014, is located in the 5′ flanking region of *SLC16A3* and predicted to have weak enhancer effects. Two other proxy SNPs for rs7503429 (rs35121878 and rs12601778) are located in intron regions of *SLC16A3*, which is 5′ of *CSNK1D*, were predicted to have weak promoter effects, either weak or strong enhancer effects, and regulatory effects on downstream and upstream TSS.

Table 3. Cumulative effect of risk genotypes in colorectal cancer

Number	Case, *N* (%)	Control, *N* (%)	OR (95% CI)[a]	*P*
0–1	66 (8.3)	100 (12.5)	1.00 (reference)	
2	257 (32.3)	334 (41.8)	1.17 (0.82–1.66)	0.39
3	284 (35.6)	232 (29.0)	1.86 (1.30–2.65)	6.7×10^{-4}
4–7	190 (23.8)	133 (16.7)	2.19 (1.49–3.21)	6.0×10^{-5}
P_{trend}				4.19×10^{-8}

[a]Adjusted for age and gender.

Discussion

Currently, only a small fraction of the known familial risk of colorectal cancer can be explained by identified genetic loci. This underscores the need to further define the full spectrum of genetic variation that contributes to colorectal cancer. Established screening guidelines have shown to be effective in detecting early-stage colorectal cancer that are curable. However, screening guidelines in the general population are hampered by the lack of informative risk markers that can improve risk assessment to identify candidates for screening beyond age.

Table 4. Associations of the Wnt/β-catenin pathway variants with colorectal cancer risk in replication and pooled populations

			Discovery		Replication		Pooled	
SNP	**Gene**	**MOI**	**OR[a] (95% CI)**	***P***	**OR[a] (95% CI)**	***P***	**OR[a] (95% CI)**	***P***
rs2545162	*APC*	rec	1.65 (1.23–2.22)	0.00090	1.15 (1.00–1.34)	0.056	1.42 (1.16–1.74)	0.00085
rs11775139	*FZD3*	rec	0.75 (0.58–0.97)	0.030	0.80 (0.69–0.93)	0.0034	0.85 (0.76–0.94)	0.0011
rs3732360	*GSK3B*	rec	0.64 (0.44–0.93)	0.018	0.86 (0.70–1.06)	0.16	0.78 (0.59–1.04)	0.088
rs17438384	*FZD3*	rec	1.63 (1.19–2.24)	0.0024	0.93 (0.79–1.08)	0.33	1.21 (0.96–1.53)	0.099
rs638051	*LRP5*	rec	1.35 (1.03–1.77)	0.029	0.92 (0.74–1.14)	0.44	1.76 (0.97–1.43)	0.10
rs624947	*LRP5*	rec	1.43 (1.05–1.96)	0.025	0.79 (0.57–1.09)	0.15	1.09 (0.94–1.26)	0.24
rs4135385	*CTNNB1*	dom	1.25 (1.03–1.53)	0.026	0.89 (0.59–1.34)	0.58	1.11 (0.96–1.28)	0.15
rs459552	*APC*	rec	1.76 (1.12–2.76)	0.014	NA	NA	NA	NA

Abbreviations: MOI, model of inheritance; rec, recessive; dom, dominant.
[a]Adjusted for age and gender.

The Wnt/ β-catenin stem cell signaling pathway has a well-established role in colorectal cancer development with rare germline mutations being responsible for a familial colorectal cancer syndrome. Therefore, there is strong biologic plausibility that common, germline genetic variants in this pathway could also modulate risk for colorectal cancer. In this study, we tested this hypothesis in a two-stage study design and identified several variants that altered risk with predicted biologically plausible functional effects.

We identified *FZD3*:rs11775139 as a highly significant variant associated with colorectal cancer risk in the discovery and replication populations, which implicates a role for this variant in the risk of colorectal cancer. Although *APC*:rs2545162 conferred an increased risk, we found *FZD3*:rs11775139 was associated with a reduced risk of colorectal cancer, implicating a polygenic effect on risk of developing colorectal cancer. Variants in high LD with *FZD3*:rs11775139 were predicted to have functional effects on transcriptional regulation; however, some of these predicted effects were weak. *FZD3*:rs164658 had *cis*-eQTL effects with *FZD3*, suggesting that it is located within a region that directly effects *FZD3* expression, potentially through modulation of methylation sites enriched in this region. As a transmembrane receptor and activator of the Wnt/β-catenin pathway, FZD3 interacts with Wnt and activates Dishevelled (DSH) to recruit other components of the destruction complex, thereby, reducing β-catenin degradation. The blocked degradation of β-catenin leads to an accumulation of β-catenin in the nucleus and upregulation of oncogenic activity (22, 31). *FZD3* is known to be overexpressed in colorectal cancer tissues and cell lines, and is highly correlated with colorectal cancer progression (31, 32).

Both main effect and gene-based analyses implicated the APC gene as a predictor of colorectal cancer risk. Although *APC*:rs2545162 was only borderline significant in the replication population and significant in the pooled population, the functional prediction highlights a potential role for APC in the risk of colorectal cancer. In our *in silico* analysis, we identified a potential *cis*-eQTL for this variant with *REEP5* (receptor expression-enhancing protein 5). *REEP5* also known as DP1 (deleted protein 1) is a member of the DP1/Yop1p protein family involved in the endoplasmic reticulum tubule formation (33). Evidence suggests that DP1 plays a tumor-suppressor role in colon tumorigenesis (34). Furthermore, several other variants in high LD with *APC*:rs2545162

Table 5. *In silico* SNPs identified in the Wnt/β-catenin pathway

Gene	**SNP**	**SNP location**	**Predicted functional effect**	**cis-eQTL (location relative to SNP)**
FZD3	rs11775139[a]	Intron	Weak enhancer; upstream and downstream TSS	—
	rs6997072	Intron	Weak enhancer	—
	rs6558067	Intron	Weak enhancer; upstream and downstream TSS	—
	rs11781193	Intron	Strong enhancer; upstream flanking TSS	—
	rs2323018	Intron	Strong enhancer; weak enhancer	—
	rs2874941	Intron	Strong enhancer; upstream flanking TSS	—
	rs164658[a]	3′ flanking region	Weak enhancer	*FZD3*
	rs1908916[a]	5′ flanking region	Active promoter; weak promoter; poised promoter	—
APC	rs2545162[a]	Intron	Strong enhancer-like	*REEP5* (53.65 kb downstream)
	rs2545164	Intron	Strong enhancer-like	—
	rs563556	Intron	Strong enhancer-like	*REEP5* (30.19 kb downstream)
	rs1734243	Intron	Strong enhancer-like	—
	rs2431238	Intron	Strong enhancer-like	—
	rs459552[a]	Missense	Missense substitution	*REEP5* (7.43 kb downstream)
CSNK1D	rs11653735[a]	Intron	Strong enhancer-like; nonsense mediated decay	*CSNK1D; CD7* (64 kb downstream); *STRA13* (227 kb upstream)
	rs7209167[a]	Intron	Weak promoter; active promoter; upstream and downstream TSS	—
	rs9901910[a]	Intron	Strong enhancer	*DCXR* (202 kb upstream)
	rs7503429[a]	*SLC16A3*/intron	Weak promoter; strong enhancer; strong upstream TSS; weak downstream TSS	*SLC16A3; DCXR* (196 kb upstream); *STRA13* (209 kb upstream)
	rs66871014	*SLC16A3*/5′-FR	Weak enhancer	—
	rs35121878	*SLC16A3*/intron	Weak promoter; weak enhancer; upstream and downstream TSS	—
	rs12601778	*SLC16A3*/intron	Weak promoter; strong enhancer	—

[a]Genotyped SNP.

(rs2545164, rs563556, rs1734243, and rs2431238) were predicted to have direct regulatory effects on *APC*, including transcriptional effects. In addition to *APC*:rs2545162, rs563556, and rs459552 in *APC* had cis-eQTL effects with *REEP5*. Although these variants are less penetrant than the rare syndromic mutations, the gene-based analysis implicated *APC* as exerting an effect on susceptibility to colorectal cancer, underscoring the importance of this gene in colorectal cancer development.

As one of the candidate genes significant in the gene-based analysis, *CSNK1D* (casein kinase 1, delta) variants were assessed for predictive functional effects, including two variants that were significant in the main effect analysis. The predicted functional effects for *CSNK1D*:rs11653735 suggested that this intronic variant may regulate mRNA expression levels for *CSNK1D* through disrupting a nonsense mediated decay signal. It was also predicted that rs11653735 is a *cis*-eQTL for *CSNK1D*, *CD7* (cluster differentiation 7), and *STRA13* (stimulated by retinoic acid 13), indicating that this variant may function in regulating the expression of these genes. Of interest is that several of these genes have been implicated in colorectal cancer development previously. *CD7* (cluster of differentiation 7) encodes for an antigen expressed on the surface of T cells, which is elevated in colorectal tumor-infiltrating lymphocytes (35). *STRA13*, a bHLH transcription factor, has been found to be elevated in colorectal cancer tumor tissue (36). Other *cis*-eQTL effects for *CSNK1D* variants include rs9901910 for *DCXR* (dicarbonyl/L-xylulose reductase) and rs7503429 for *SLC16A3*, *DCXR*, and *STRA13*. Although several variants in our study were predicted to have direct functional effects on expression of the aforementioned genes, it has been reported that mutations in *CSNK1D*, encoding for a serine–threonine protein component of the β-catenin destruction complex, can promote the development of colorectal cancer (37). *CSNK1D* is located close to *SLC16A3*, encoding a monocarboxylate transporter, with potential for overlapping regulatory elements. Several of the variants linked with *CSNK1D*, rs7503429, rs35121878, and rs12601778, are potentially located within overlapping regulatory elements in *SLC16A3* intron regions, thus making it difficult to determine whether the variants are functioning on the regulation of *CSNK1D*, *SLC16A3*, or both.

The major strength of this study was the two-stage study design that included a total of 1,502 cases and 1,589 controls. When performing genetic association analyses, false positives are to be expected, which underscores the value of having a replication set and performing a pooled analysis in our study. Coupled with our bootstrap resampling results, we have increased our confidence that our significant findings are not due to random chance. Furthermore, layering potential functional effects through *in silico* prediction provides biologic plausibility to our observed genetic associations. Several of the predicted functional elements were regulatory in nature, suggesting that luciferase reporter assays using colorectal cancer cell lines could potentially measure the effect of variants on function and link genetic variation to phenotypic variation.

Although we adjusted for gender in our analyses, the excess males in the discovery population could be a source for potential gender imbalance effects which might have had an impact on the number of variants that were replicated. External replication, including analysis for potential gender-specific effects, is warranted to further define the effect of these variants. External replication could also expand the findings to other racial/ethnic populations. In addition, we selected the top eight most promising SNPs from our discovery to move into the replication phase to try to minimize false-positive associations. In parallel, there may be additional variants that were significant in our discovery that are true positives and worthy of further analysis.

Moving these findings into clinical and translational application would require assessment of these variants within the context of established colorectal cancer risk factors, such as family history, personal history of colorectal cancer/adenomas, diet, and others that are emerging. Future studies are needed to determine the increase in prediction conferred by these variants in studies that have these variables available for a large number of study participants. Improved risk assessment would enable risk-stratified screening recommendations. For example, current guidelines recommend screening based on age, resulting in many individuals >50 years of age who are at low risk undergoing unnecessary screening, whereas high-risk individuals <50 years of age are not screened in the absence of a strong family history. Colorectal cancer in this younger age group is increasing in incidence (38), suggesting that more refined risk assessment is needed in this population.

In summary, our study suggests that multiple common germline SNPs in components of the Wnt/β-catenin pathway maybe associated with colorectal cancer risk. Specifically, *FZD3* and *APC* genetic variations, including functional variants in high LD with *FZD3*:rs11775139, may increase susceptibility to colorectal cancer with strong biologic plausibility, as demonstrated by our *in silico* functional prediction analysis. The findings from this study underscore the importance of the Wnt/β-catenin pathway in helping to identify those at increased risk for colorectal cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

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Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): M.A.T. Hildebrandt, M.E. Reyes, M. Lin, Y. He, S.V. Nguyen, X. Wu

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Grant Support

The funding of this study was provided by a Duncan Family Institute for Cancer Prevention and Risk Assessment Seed Grant (to M.A.T. Hildebrandt). Additional support provided by the Center for Translational and Public Health Genomics of the Duncan Family Institute and by the NIH through MD Anderson's Cancer Center Support Grant CA016672.

Received August 6, 2015; revised December 14, 2015; accepted January 6, 2016; published OnlineFirst January 25, 2016.

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