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Original article

Genetic variants in the WNT signaling pathway are protectively associated with colorectal cancer in a Saudi population

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ABSTRACT

The Wnt/β-catenin signaling pathway has been etiologically implicated in the development and progression of colorectal cancer. We studied thirteen single nucleotide polymorphisms (SNPs) located in SFRP3 (rs7775), CTNNB1 (β-catenin) [rs4135385, rs13072632], APC (rs454886, rs459552), LRP6 (rs2075241, rs2284396), DKK4 (rs3763511), DKK3 (rs6485350), TCF4 (rs12255372) and AXIN2 (rs3923086, rs3923087, rs4791171) in patients with colorectal cancer (n = 122) and controls (n = 110). Evaluation of WNT pathway SNPs showed protective association for rs4135385, located in β-catenin. Additionally, variants in SFRP3 (rs7775) and LRP6 (rs2284396) which did not show any association in the overall analysis were significantly associated with female and old aged colorectal cancer patients, respectively.

1. Introduction

Colorectal cancer (CRC) is the second most common cause of cancer related mortalities in Saudi Arabia, and the incidence is rising since past decade. Accumulated evidence in the past three decades suggests WNT signaling pathway genes to be mutated in very high proportion of sporadic colorectal tumors. Wnt ligands are a family of 19 glycoproteins which have a key role in early development and tissue homeostasis. Any changes in WNT signaling genes may cause disease including colorectal cancer (Mao et al., 2001; Segditsas and Tomlinson, 2006). The possible role of WNT genes in cancer has been reported few decades ago in mouse models. Variation in expression levels of WNT1 lead to tumor formation in transgenic mice (Nusse et al., 1984). Further studies reported that WNT genes promoted stabilization of β-catenin and β-catenin dependent transcription. Axin, APC and GSK3β forms β-catenin destruction complex. Canonical WNT pathway activity is dependent on this complex, which eradicates newly formed β-catenin protein through the ubiquitin–proteasome pathway in the off-state when WNT ligands are not bound to its receptors frizzled and LRP5/6 (Benham-Pyle et al., 2016). In the on-state, WNT ligands bind to its receptors resulting in cytoplasmic accumulation of β-catenin which than translocates into the nucleus leading to formation of a complex with TCF/LEF family of transcription factors. This complex formation drives transcriptional activation of genes involved in cell proliferation such as c-Myc and Cyclin D (Clevers, 2006).

The high rate of WNT pathway genes mutations in various cancers emphasizes the significance of WNT/β-catenin signaling pathway in cancer progression. Apart from APC that has been reported to play crucial role in colorectal cancer progression, The Cancer Genome Atlas Network has reported the involvement of several other WNT pathway genes (Anastas and Moon, 2013). Although mutations in genes such as FZD4, LRP5 and LRP6 that obstruct WNT signaling have been recognized in other diseases, similar WNT-pathway inactivating mutations have not been identified in cancer (Anastas and Moon, 2013). Most of the WNT pathway gene mutations reported in cancer are found to result in hyperactivation of WNT pathway. β-catenin missense and other mutations are very common in hepatocellular carcinoma and ovarian cancer, whereas deletions and truncation mutations in *AXIN1* are commonly

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observed in hepatocellular carcinoma and colorectal cancers (Giles et al., 2003;Anastas and Moon, 2013). Thus, in light of the previous reports that provide compelling evidence of the involvement of Wnt signaling pathway in the progression of colorectal cancer, we in the present study evaluated the association of SNPs in WNT signaling pathway genes with susceptibility to colorectal cancer in Saudi population. We investigated 13 germline polymorphisms in 8 genes involved in the Wnt signaling pathway to evaluate their risk association in patients with colorectal cancer.

2. Materials and methods

2.1. Study population

This study was approved by ethical review committee of King Khalid University Hospital, Riyadh, Saudi Arabia and written informed consent was obtained from all participants. The samples comprised of 122 colorectal cancer patients (age 18–82 years, mean age 57 years). Additionally, 110 gender and age matched non-cancer samples were recruited as controls in the present study. 5 ml blood was collected from patients and healthy individuals. Histology and TNM staging were analyzed to verify the diagnosis. Demographic information for the colorectal cancer and control subjects are presented in Table 1.

2.2. Genotyping

The DNA was isolated from colorectal cancer patients and control blood samples using DNA extraction kit (Qiagen, Valencia, CA) according to the manual of manufacturer. A total of 13 SNPs located in eight genes involved in WNT signaling pathway were selected based on previous literature and genotyped using predesigned TaqMan assays using ABI 7500 real-time PCR machine (Applied Biosystems, USA) as previously described (Almutairi et al., 2015). The SNPs selected were located in SFRP3 (rs7775), APC (rs454886, rs459552), LRP6 (rs2075241, rs2284396), DKK4 (rs3763511), AXIN2 (rs3923086, rs3923087, rs4791171), β-catenin (rs4135385, rs13072632), DKK3 (rs6485350) and TCF4 (rs12255372) genes respectively. Around 10% of the samples were randomly used to reconfirm the results.

2.3. Statistical analysis

The Hardy–Weinberg equilibrium was assessed using $\chi 2$ test for controls and cases. Pearson's goodness-of-fit chi-square ($\chi 2$) values, odds ratios (OR), 95% confidence intervals (CI), and p values were calculated using SPSS *ver* 22 to find out the association between genotypes of all the SNPs with colorectal cancer risk as described by Alanazi et al. (2013).

3. Results

The demographic characteristics of selected samples are shown in Table 1.

Table 1
Demographic characteristics of CRC patients and control subjects.

Characteristics	Case	Control
Samples	122	110
Age		
<57	62	48
>57	60	62
Gender		
Male	74	57
Female	48	53

3.1. Association of SNPs with colorectal cancer risk

The study group comprised of 122 patients with histopathologically confirmed colorectal cancer and 110 age and gender matched cancer-free controls (Table 1). To evaluate the role of WNT pathway genes in colorectal carcinogenesis, we examined 13 SNPs in eight genes of WNT signaling pathway (Table 2).

Ancestral allele was selected based on NCBI SNP database and used as a reference to calculate the odds to check the association of genotypes and alleles with colorectal cancer. The overall genotype frequencies of the analyzed SNPs and the odds ratio and significance are presented in Table 2. The allelic frequencies of all tested SNPs were in limits of Hardy–Weinberg equilibrium. The homozygous GG genotype of SNP rs4135385 in the β-catenin gene showed significant protective association (OR: 0.092, p = 0.03) (Table 2). We did not detect any statistically significant association with the risk of developing colorectal cancer for the other twelve SNPs examined in the overall study population (Table 2). However, when the samples were segregated based on gender and age at disease diagnosis, SNP rs7775 in SFRP3 gene showed significant protective association in female patients with minor allele Gly (OR 0.397, p = 0.02) as well as with heterozygous Arg/Gly genotype (OR 0.408, p = 0.04 (Table 3). None of the evaluated SNPs showed significant protective or risk association with colorectal cancer in males (Table 4) as well as in patients whose age at the time of disease diagnosis was below 57 years (Table 5). Interestingly, the CC genotype of LRP6 gene SNP rs2284396 showed significant protective association with colorectal cancer in patients who were above 57 years of age at the time of disease diagnosis (OR: 0.250, p = 0.021). In the allelic model as well similar protection against colorectal cancer was observed with the C allele of LRP6 SNP rs2284396 in individuals who were above 57 years of age (OR 0.561, p = 0.03) (Table 6).

4. Discussion

The present study evaluated the association of WNT signaling pathway gene variants with colorectal cancer susceptibility in Saudi population. Three of the 13 SNPs that were examined in this study showed significant decreased risk association with colorectal cancer. Two of the three protectively associated SNPs were found to be in the intron region and only SFRP3 gene SNP rs7775 was in the exon that codes for either Arg (CGC) or Gly (GGC). We found a strong association of the β-catenin gene rs4135385 with a decreased CRC risk. It was observed that individuals carrying GG genotype have approximately 11-fold lower risk of developing colorectal cancer relative to those having AA genotype at rs4135385 of β-catenin. This is in accordance with Wang et al. who investigated the rs4135385 and identified significant association of increased gastric cancer risk in Chinese patients having AG genotype compared to those having GG genotype (Wang et al., 2012). In our previous study as well we found significant risk association with rs4135385 in breast cancer while the other SNP rs13072632 in β-catenin was not associated (Alanazi et al., 2013). Zhang et al. reported that there is no association between rs4135385 and acute leukemia (Zhang et al., 2015).

LRP6 gene SNP rs2284396 showed decreased risk of colorectal cancer in above 57 years old patients with CC genotype and C allele. A fourfold decreased risk of developing CRC was observed in individuals with CC genotype compared to those having TT genotype at rs2284396. SNP rs2284396 as well as other SNPs in LRP6 didn't show any association in diabetes mellitus in Japanese population (Zenibayashi et al., 2008). However, Bai et al. reported an association of LRP6 SNP rs2284396 with Alzheimer's disease (Bai et al., 2016).

Table 2
Genotype frequencies of WNT pathway gene polymorphism in colorectal cancer cases and controls.

Gene	SNP	Variant	Cases (Freq)	Controls	OR	CI	χ^2 Value	P-Value
SFRP3	rs7775	Arg/Arg	96 (0.79)	77(0.70)	Ref			
		Arg/Gly	24 (0.20)	30(0.27)	0.642	0.347–1.187	2.02	0.15570
		Gly/Gly	2 (0.01)	3 (0.02)	0.535	0.087–3.281	0.47	0.49239
		Arg	216(0.89)	184(0.83)	Ref			
		Gly	28 (0.11)	36 (0.16)	0.663	0.389–1.127	2.32	0.12732
APC	rs454886	TT	53 (0.45)	53(0.49)	Ref			
		TC	54 (0.46)	44 (0.40)	1.227	0.707–2.129	0.53	0.46598
		CC	10 (0.9)	13 (0.11)	0.769	0.310–1.907	0.32	0.57058
		T	160(0.68)	150(0.69)	Ref			
		C	74 (0.32)	70 (0.31)	0.991	0.667–1.472	0.00	0.96455
APC	rs459552	Val/Val	88 (0.72)	72 (0.65)	Ref			
		Val/Asp	30 (0.25)	35 (0.32)	0.701	0.393–1.251	1.45	0.22848
		Asp/Asp	4 (0.33)	3 (0.02)	1.091	0.236–5.033	0.01	0.91117
		Val	206(0.84)	179(0.81)	Ref			
		Asp	38 (0.16)	41 (0.19)	0.805	0.496–1.308	0.77	0.38080
LRP6	rs2075241	GG	92 (0.75)	76 (0.70)	Ref			
		GC	24 (0.19)	31 (0.29)	0.640	0.346–1.181	2.05	0.15172
		CC	6 (0.04)	1 (0.009)	4.957	0.584–42.073	2.61	0.10600
		G	208(0.85)	183(0.85)	Ref			
		C	36 (0.14)	33 (0.15)	0.960	0.575–1.602	0.02	0.87526
LRP6	rs2284396	TT	64 (0.53)	50 (0.46)	Ref			
		TC	43 (0.35)	40 (0.37)	0.840	0.476–1.482	0.36	0.54661
		CC	14 (0.11)	19 (0.17)	0.576	0.263–1.260	1.93	0.16442
		T	171(0.70)	140(0.64)	Ref			
		C	71 (0.3)	78 (0.36)	0.745	0.504–1.102	2.17	0.14049
DKK4	rs3763511	CC	87 (0.70)	80 (0.73)	Ref			
		CT	32 (0.26)	29 (0.26)	1.015	0.564–1.825	0.00	0.96124
		TT	4 (0.03)	1 (0.009)	3.678	0.403–33.604	1.52	0.21805
		C	206(0.83)	189(0.86)	Ref			
		T	40 (0.16)	31 (0.14)	1.184	0.712–1.969	0.42	0.51536
AXIN2	rs3923086	TT	48 (0.39)	41 (0.37)	Ref			
		TG	52 (0.42)	50 (0.45)	0.888	0.503–1.570	0.17	0.68364
		GG	21 (0.17)	19 (0.17)	0.944	0.447–1.994	0.02	0.88007
		T	148(0.61)	132(0.60)	Ref			
		G	94 (0.38)	88 (0.40)	0.953	0.656–1.384	0.06	0.79934
AXIN2	rs3923087	AA	35 (0.28)	37 (0.33)	Ref			
		AG	56 (0.45)	50 (0.45)	1.184	0.650–2.156	0.31	0.58049
		GG	32 (0.26)	23 (0.20)	1.471	0.725–2.984	1.15	0.28440
		A	126(0.51)	124(0.56)	Ref			
		G	120(0.49)	96 (0.44)	1.230	0.854–1.773	1.24	0.26627
AXIN2	rs4791171	AA	40 (0.32)	38 (0.34)	Ref			
		AG	55 (0.45)	48 (0.43)	1.089	0.604–1.962	0.08	0.77771
		GG	27 (0.22)	24 (0.22)	1.069	0.527–2.167	0.03	0.85370
		A	135(0.55)	124(0.56)	Ref			
		G	109(0.44)	96 (0.44)	1.043	0.723–1.505	0.05	0.82250
β-catenin	rs4135385	AA	89 (0.74)	74 (0.67)	Ref			
		AG	31 (0.26)	32 (0.30)	0.805	0.450–1.442	0.53	0.46618
		GG	0 (0.0)	4 (0.04)	0.092	0.005–1.746	4.68	**0.03059**
		A	209(0.87)	180(0.81)	Ref			
		G	31 (0.13)	40 (0.18)	0.667	0.401–1.111	2.44	0.11844
β-catenin	rs13072632	CC	51 (0.42)	46 (0.42)	Ref			
		CT	47 (0.39)	51 (0.46)	0.831	0.474–1.458	0.42	0.51900
		TT	23 (0.19)	13 (0.11)	1.596	0.725–3.510	1.36	0.24335
		C	149(0.61)	143(0.65)	Ref			
		T	93 (0.38)	77 (0.35)	1.159	0.793–1.694	0.58	0.44519
DKK3	rs6485350	AA	46 (0.39)	36 (0.33)	Ref			
		AG	56 (0.47)	50 (0.45)	0.877	0.491–1.564	0.20	0.65563
		GG	17 (0.14)	24 (0.22)	0.554	0.259–1.184	2.34	0.12586
		A	148(0.62)	122(0.55)	Ref			
		G	90 (0.37)	98 (0.44)	0.757	0.521–1.100	2.14	0.14350
TCF4	rs12255372	GG	47 (0.40)	49 (0.44)	Ref			
		GT	56 (0.47)	46 (0.42)	1.269	0.726–2.219	0.70	0.40279
		TT	17 (0.14)	15 (0.14)	1.182	0.530–2.633	0.17	0.68309
		G	150(0.62)	144(0.65)	Ref			
		T	90 (0.37)	76 (0.34)	1.137	0.776–1.665	0.43	0.50982

SFRP3 gene showed significant protective association in female patients harbouring minor allele G. The G allele of rs7775 codes for Gly while the C allele codes for Arg. Women having Gly at codon 324 (rs7775) of SFRP3 have 2.5-fold lower risk of developing CRC relative to those have Arg at this locus. Our finding of the strong protection conferred by the GG genotype of rs7775 against

Table 3
Distribution of WNT pathway gene SNPs genotype and allele frequencies in colorectal cancer cases and control population based on gender (female).

SNP	Variant	Cases (Freq)	Controls	OR	CI	χ^2 Value	P-Value
rs7775	Arg/Arg	38 (0.79)	31 (0.59)	Ref			
	Arg/Gly	10 (0.20)	20 (0.38)	0.408	0.167–0.998	3.96	**0.04670**
	Gly/Gly	0 (0.0)	2 (0.03)	0.164	0.008–3.534	2.37	0.12370
	Arg	86 (0.90)	82 (0.80)	Ref			
	Gly	10 (0.10)	24 (0.20)	0.397	0.179–0.882	5.38	**0.02039**
rs454886	TT	24 (0.55)	26 (0.49)	Ref			
	CT	17 (0.40)	21 ()0.40	0.877	0.376–2.045	0.09	0.76116
	CC	2 (0.05)	6 (0.11)	0.361	0.066–1.964	1.48	0.22454
	T	65 (0.76)	73 (0.69)	Ref			
	C	21 (0.24)	33 (0.31)	0.715	0.376–1.357	1.06	0.30354
rs459552	Val/Val	35 (0.73)	35 (0.67)	Ref			
	Val/Asp	9 (0.19)	17 (0.32)	0.529	0.208–1.347	1.81	0.17882
	Asp/Asp	4 (0.08)	1 (0.01)	4.000	0.425–37.605	1.68	0.19457
	Val	79 (0.82)	87 (0.82)	Ref			
	Asp	17 (0.17)	19 (0.18)	0.985	0.479–2.028		0.96802
rs2075241	GG	41(0.85)	39 (0.73)	Ref			
	GC	5 (0.10)	13 (0.25)	0.366	0.119–1.122	3.25	0.07141
	CC	2 (0.04)	1 (0.02)	1.902	0.166–21.830	0.28	0.59983
	G	87(0.91)	91(0.86)	Ref			
	C	9 (0.09)	15 (0.14)	0.628	0.261–1.509	1.10	0.29481
rs2284396	TT	26 (0.55)	24 (0.45)	Ref			
	TC	13 (0.28)	21 (0.40)	0.571	0.235–1.387	1.54	0.21437
	CC	8 (0.17)	8 (0.15)	0.923	0.299–2.846	0.02	0.88919
	T	65 (0.70)	69 (0.65)	Ref			
	C	29 (0.30)	37 (0.35)	0.832	0.460–1.505	0.37	0.54277
rs3763511	CC	32 (0.66)	39 (0.74)	Ref			
	CT	14 (0.30)	14 (0.26)	1.219	0.508–2.926	0.20	0.65783
	TT	2 (0.04)	0 (0.0)	6.077	0.282–131.123	2.36	0.12458
	C	78 (0.81)	92 (0.87)	Ref			
	T	18 (0.19)	14 (0.13)	1.516	0.709–3.245	1.16	0.28131
rs3923086	TT	19 (0.40)	19 (036)	Ref			
	TG	21 (0.44)	24 (0.45)	0.875	0.369–2.077	0.09	0.76204
	GG	8 (0.16)	10 (0.19)	0.800	0.259–2.468	0.15	0.69759
	T	59 (0.61)	62 (0.59)	Ref			
	G	37 (0.39)	44 (0.41)	0.884	0.503–1.553	0.18	0.66735
rs3923087	AA	14 (0.29)	18 (0.34)	Ref			
	AG	21 (0.43)	23 (0.43)	1.174	0.470–2.932	0.12	0.73126
	GG	13 (0.27)	12 (0.23)	1.393	0.487–3.982	0.38	0.53591
	A	49 (0.51)	59 (0.56)	Ref			
	G	47 (0.49)	47 (0.44)	1.204	0.692–2.095	0.43	0.51104
rs4791171	AA	15 (0.32)	19 (0.36)	Ref			
	AG	26 (0.55)	19 (0.36)	1.733	0.705–4.259	1.45	0.22891
	GG	6 (0.28)	15 (0.28)	0.507	0.158–1.623	1.33	0.24894
	A	56 (0.60)	57 (0.54)	Ref			
	G	38 (0.40)	49 (0.46)	0.789	0.450–1.384	0.68	0.40886
rs4135385	AA	34 (0.74)	34 (0.64)	Ref			
	AG	12 (0.26)	16 (0.30)	0.750	0.309–1.820	0.41	0.52428
	GG	0 (0.0)	3 (0.06)	0.143	0.007–2.871	2.88	0.08978
	A	80 (0.87)	84 (0.79)	Ref			
	G	12 (0.13)	22 (0.21)	0.573	0.266–1.233	2.06	0.15130
rs13072632	CC	22 (0.46)	22 (0.42)	Ref			
	CT	16 (0.33)	25 (0.47)	0.640	0.270–1.515	1.03	0.30916
	TT	10 (0.21)	6 (0.11)	1.667	0.516–5.381	0.74	0.39075
	C	60 (0.63)	69 (0.65)	Ref			
	T	36 (0.37)	37 (0.35)	1.119	0.630–1.988	0.15	0.70150
rs6485350	AA	19 (0.43)	16 (0.30)	Ref			
	AG	19 (0.43)	29 (0.55)	0.552	0.229–1.332	1.76	0.18429
	GG	6 (0.14)	8 (0.15)	0.632	0.181–2.205	0.52	0.46971
	A	57 (0.65)	61 (0.58)	Ref			
	G	31 (0.35)	45 (0.42)	0.737	0.412–1.320	1.05	0.30470
rs12255372	GG	20 (0.42)	22 (0.42)	Ref			
	GT	18 (0.37)	25 (0.47)	0.792	0.336–1.865	0.29	0.59342
	TT	10 (0.21)	6 (0.11)	1.833	0.564–5.963	1.03	0.31075
	G	58 (0.60)	69 (0.65)	Ref			
	T	38 (0.40)	37 (0.35)	1.222	0.690–2.164	0.47	0.49198

colorectal cancers however in a small population size is significant and provides strong reason for examination of this SNP in larger studies in other ethnic groups. In our previous study we found that the CG and GG genotypes of rs7775 were protectively associated with breast cancers (Alanazi et al., 2013). Our results are in contrast with the findings of Shanmugam et al. (2007) who reported

Table 4
Distribution of WNT pathway gene SNPs genotype and allele frequencies in colorectal cancer cases and control population based on gender (male).

SNP	Variant	Cases (Freq)	Controls	OR	CI	χ^2 Value	P-Value
rs7775	Arg/Arg	58 (0.78)	46(0.81)	Ref			
	Arg/Gly	14 (0.19)	10(0.18)	1.110	0.452–2.728	0.05	0.81946
	Gly/Gly	2 (0.03)	1(0.01)	1.586	0.139–18.044	0.14	0.70770
	Arg	130(0.88)	102(0.89)	Ref			
	Gly	18(0.12)	12(0.11)	1.177	0.542–2.555	0.17	0.68015
rs454886	TT	29(0.39)	27(0.47)	Ref			
	CT	37(0.50)	23(0.40)	1.498	0.716–3.135	1.15	0.28289
	CC	8(0.11)	7(0.13)	1.064	0.340–3.333	0.01	0.91514
	T	95(0.64)	77(0.68)	Ref			
	C	53(0.36)	37(0.32)	1.161	0.693–1.946	0.32	0.57079
rs459552	Val/Val	53(0.72)	37(0.65)	Ref			
	Val/Asp	21(0.28)	18(0.32)	0.814	0.382–1.736	0.28	0.59481
	Asp/Asp	0(0.0)	2(0.03)	0.140	0.007–3.005	2.78	0.09555
	Val	127(0.86)	92(0.81)	Ref			
	Asp	21(0.14)	22(0.19)	0.691	0.359–1.332	1.23	0.26833
rs2075241	GG	51(0.69)	37(0.67)	Ref			
	GC	19(0.26)	18(0.33)	0.766	0.354–1.656	0.46	0.49719
	CC	4(0.05)	0(0.0)	6.553	0.342–125.445	2.81	0.09349
	G	121(0.82)	92(0.84)	Ref			
	C	27(0.18)	18(0.16)	1.140	0.592–2.196	0.15	0.69397
rs2284396	TT	38(0.52)	26(0.46)	Ref			
	TC	30(0.40)	19(0.34)	1.080	0.505–2.312	0.04	0.84224
	CC	6(0.08)	11(0.20)	0.373	0.123–1.136	3.14	0.07644
	T	106(0.72)	71(0.63)	Ref			
	C	42(0.28)	41(0.37)	0.686	0.406–1.160	1.99	0.15872
rs3763511	CC	55(0.73)	41(0.72)	Ref			
	CT	18(0.24)	15(0.26)	0.895	0.404–1.982	0.08	0.78364
	TT	2(0.03)	1(0.02)	1.491	0.131–17.008	0.10	0.74629
	C	128(0.85)	97(0.85)	Ref			
	T	22(0.15)	17(0.15)	0.981	0.494–1.947	0.00	0.95557
rs3923086	TT	29(0.40)	22(0.39)	Ref			
	TG	31(0.42)	26(0.45)	0.905	0.423–1.936	0.07	0.79595
	GG	13(0.18)	9(0.16)	1.096	0.397–3.022	0.03	0.85972
	T	89(0.61)	70(0.61)	Ref			
	G	57(0.39)	44(0.39)	1.019	0.616–1.684	0.01	0.94182
rs3923087	AA	21(0.28)	19(0.33)	Ref			
	AG	35(0.47)	27(0.47)	1.173	0.528–2.606	0.15	0.69536
	GG	19(0.25)	11(0.20)	1.563	0.594–4.113	0.82	0.36473
	A	77(0.51)	65(0.57)	Ref			
	G	73(0.49)	49(0.43)	1.258	0.771–2.053	0.84	0.35884
rs4791171	AA	25(0.33)	19(0.33)	Ref			
	AG	29(0.39)	29(0.51)	0.760	0.346–1.671	0.47	0.49444
	GG	21(0.28)	9(0.16)	1.773	0.664–4.737	1.32	0.25099
	A	79(0.53)	67(0.59)	Ref			
	G	71(0.47)	47(0.41)	1.281	0.784–2.095	0.98	0.32301
rs4135385	AA	55(0.74)	40(0.70)	Ref			
	AG	19(0.26)	16(0.28)	0.864	0.396–1.884	0.14	0.71243
	GG	0(0.0)	1(0.02)	0.243	0.010–6.125	1.36	0.24430
	A	129(0.87)	96(0.84)	Ref			
	G	19(0.13)	18(0.16)	0.786	0.391–1.577	0.46	0.49641
rs13072632	CC	29(0.40)	24(0.42)	Ref			
	CT	31(0.42)	25(0.44)	1.026	0.482–2.183	0.00	0.94646
	TT	13(0.18)	8(0.14)	1.345	0.478–3.780	0.32	0.57366
	C	89(0.61)	73(0.64)	Ref			
	T	57(0.39)	41(0.36)	1.140	0.687–1.893	0.26	0.61154
rs6485350	AA	27(0.36)	20(0.35)	Ref			
	AG	37(0.49)	21(0.37)	1.305	0.593–2.870	0.44	0.50745
	GG	11(0.14)	16(0.28)	0.509	0.195–1.331	1.92	0.16632
	A	91(0.60)	61(0.54)	Ref			
	G	59(0.40)	53(0.46)	0.746	0.456–1.221	1.36	0.24377
rs12255372	GG	27(0.37)	27(0.47)	Ref			
	GT	38(0.53)	21(0.37)	1.810	0.851–3.846	2.39	0.12172
	TT	7(0.10)	9(0.16)	0.778	0.253–2.390	0.19	0.66042
	G	92(0.64)	75(0.66)	Ref			
	T	52(0.36)	39(0.34)	1.087	0.649–1.819	0.10	0.75103

that variations in rs7775 significantly increased risk for CRC in German patients (Shanmugam et al., 2007). Few other studies found no association of this SNP with colorectal cancer (Berndt et al., 2009) and osteoarthritis (Loughlin et al., 2004). Thus, it may be possible that rs7775 brings about different outcome in cooperation with other SNPs and warrants a detailed investigation

Table 5
Distribution of WNT pathway gene SNPs genotype and allele frequencies in colorectal cancer cases and control population based on age (<57).

SNP	Variant	Cases (Freq)	Controls	OR	CI	χ^2 Value	P-Value
rs7775	Arg/Arg	48(0.78)	37(0.77)	Ref			
	Arg/Gly	13(0.21)	9(0.19)	1.113	0.430–2.885	0.05	0.82488
	Gly/Gly	1(0.01)	2(0.41)	0.385	0.034–4.415	0.63	0.42786
	Arg	109(0.88)	83(0.86)	Ref			
	Gly	15(0.12)	13(0.14)	0.879	0.396–1.947	0.10	0.74980
rs454886	TT	25(0.44)	24(0.50)	Ref			
	CT	25(0.44)	20(0.42)	1.200	0.533–2.703	0.19	0.65979
	CC	7(0.12)	4(0.08)				
	T	75(0.66)	68(0.71)	Ref			
	C	39(0.34)	28(0.29)	1.263	0.703–2.269	0.61	0.43469
rs459552	Val/Val	43(0.68)	31(0.65)	Ref			
	Val/Asp	17(0.27)	14(0.29)	0.875	0.376–2.037	0.10	0.75747
	Asp/Asp	3(0.05)	3(0.06)	0.721	0.136–3.813	0.15	0.69920
	Val	103(0.82)	76(0.79)	Ref			
	Asp	23(0.18)	20(0.21)	0.849	0.435–1.656	0.23	0.62996
rs2075241	GG	48(0.76)	37(0.77)	Ref			
	GC	12(0.19)	10(0.21)	0.925	0.360–2.374	0.03	0.87118
	CC	3(0.04)	1(0.02)	2.312	0.231–23.145	0.54	0.46406
	G	108(0.86)	84(0.87)	Ref			
	C	18(0.14)	12(0.13)	1.167	0.533–2.556	0.15	0.69982
rs2284396	TT	32(0.52)	24(0.50)	Ref			
	TC	19(0.31)	18(0.37)	0.792	0.344–1.823	0.30	0.58279
	CC	10(0.16)	6(0.13)	1.250	0.399–3.917	0.15	0.70148
	T	83(0.68)	66(0.69)	Ref			
	C	39(0.32)	30(0.31)	1.034	0.581–1.838	0.01	0.91001
rs3763511	CC	45(0.71)	34(0.71)	Ref			
	CT	17(0.27)	13(0.27)	0.988	0.423–2.308	0.00	0.97781
	TT	1(0.01)	1(0.02)	0.756	0.046–12.517	0.04	0.84438
	C	107(0.85)	81(0.84)	Ref			
	T	19(0.15)	15(0.16)	0.959	0.459–2.002	0.01	0.91095
rs3923086	TT	27(0.13)	18(0.37)	Ref			
	TG	22(0.35)	21(0.44)	0.698	0.300–1.625	0.70	0.40417
	GG	14(0.22)	9(0.19)	1.037	0.371–2.899	0.00	0.94472
	T	76(0.60)	57(0.60)	Ref			
	G	50(0.40)	39(0.40)	0.962	0.559–1.653	0.02	0.88712
rs3923087	AA	15(0.24)	16(0.33)	Ref			
	AG	32(0.51)	24(0.50)	1.422	0.589–3.433	0.62	0.43258
	GG	16(0.25)	8(0.17)	2.133	0.708–6.428	1.84	0.17521
	A	62(0.49)	56(0.58)	Ref			
	G	64(0.51)	40(0.42)	1.445	0.846–2.468	1.82	0.17698
rs4791171	AA	22(0.35)	17(0.35)	Ref			
	AG	27(0.44)	23(0.48)	0.907	0.391–2.107	0.05	0.82058
	GG	13(0.21)	8(0.17)	1.256	0.424–3.714	0.17	0.68052
	A	71(0.57)	57(0.60)	Ref			
	G	53(0.43)	39(0.40)	1.091	0.635–1.874	0.10	0.75223
rs4135385	AA	42(0.70)	33(0.69)	Ref			
	AG	18(0.30)	13(0.27)	1.088	0.467–2.537	0.04	0.84532
	GG	0(0.0)	2(0.04)	0.158	0.007–3.396	2.46	0.11648
	A	102(0.85)	79(0.82)	Ref			
	G	18(0.15)	17(0.18)	0.820	0.397–1.693	0.29	0.59143
rs13072632	CC	23(0.37)	16(0.33)	Ref			
	CT	27(0.44)	25(0.52)	0.751	0.325–1.738	0.45	0.50348
	TT	12(0.19)	7(0.16)	1.193	0.385–3.690	0.09	0.75985
	C	73(0.59)	57(0.59)	Ref			
	T	51(0.41)	39(0.41)	1.021	0.594–1.756	0.01	0.93989
rs6485350	AA	25(0.42)	18(0.38)	Ref			
	AG	25(0.42)	18(0.37)	1.000	0.425–2.356	0.00	1
	GG	10(0.16)	12(0.25)	0.600	0.213–1.689	0.94	0.33168
	A	75(0.62)	54(0.56)	Ref			
	G	45(0.38)	42(0.44)	0.771	0.446–1.333	0.87	0.35204
rs12255372	GG	22(0.35)	23(0.48)	Ref			
	GT	29(0.47)	15(0.31)	2.021	0.860–4.750	2.63	0.10459
	TT	11(0.18)	10(0.21)	1.150	0.408–3.243	0.07	0.79157
	G	73(0.59)	61(0.64)	Ref			
	T	51(0.41)	35(0.36)	1.218	0.704–2.107	0.50	0.48137

Table 6
Distribution of WNT pathway gene SNPs genotype and allele frequencies in colorectal cancer cases and control population based on age (>57).

SNP	Variant	Cases (Freq)	Controls	OR	CI	χ^2 Value	P-Value
rs7775	Arg/Arg	48 (0.80)	40 (0.65)	Ref			
	Arg/Gly	11 (0.18)	21(0.34)	0.437	0.188–1.013	3.82	0.05064
	Gly/Gly	1 (0.02)	1 (0.01)	0.833	0.051–13.750	0.02	0.89844
	Arg	107(0.89)	101 (0.81)	Ref			
	Gly	13 (0.11)	23 (0.19)	0.534	0.256–1.110	2.89	0.08935
rs454886	TT	28(0.47)	29 (0.47)	Ref			
	CT	29 (0.48)	24 (0.39)	1.251	0.591–2.649	0.34	0.55739
	CC	3 (0.05)	9 (0.14)	0.345	0.085–1.409	2.33	0.12679
	T	85 (0.71)	82 (0.67)	Ref			
	C	35 (0.29)	42 (0.33)	0.804	0.468–1.382	0.62	0.42925
rs459552	Val/Val	45 (0.76)	41 (0.66)	Ref			
	Val/Asp	13 (0.22)	21 (0.34)	0.564	0.251–1.269	1.94	0.16397
	Asp/Asp	1 (0.02)	0(0.0)	2.736	0.108–69.043	0.90	0.34233
	Val	103(0.87)	103(0.83)	Ref			
	Asp	15 (0.13)	21 ()0.17	0.714	0.349–1.463	0.85	0.35605
rs2075241	GG	44 (0.75)	39 (0.65)	Ref			
	GC	12 (0.20)	21 (0.35)	0.506	0.221–1.162	2.62	0.10546
	CC	3 (0.05)	00.0	6.213	0.311–124.055	2.58	0.10827
	G	100(0.85)	99 (0.82)	Ref			
	C	18 (0.15)	21 (0.18)	0.849	0.426–1.689	0.22	0.63980
rs2284396	TT	32 (0.53)	26 (0.43)	Ref			
	TC	24 (0.40)	22 (0.36)	0.886	0.408–1.926	0.09	0.76063
	CC	4 (0.07)	13 (0.21)	0.250	0.073–0.859	5.27	**0.02165**
	T	88 (0.73)	74 (0.60)	Ref			
	C	32 (0.27)	48 (0.40)	0.561	0.325–0.966	4.39	**0.03607**
rs3763511	CC	42 (0.70)	46 (0.74)	Ref			
	CT	15 (0.25)	16 (0.26)	1.027	0.453–2.330	0.00	0.94958
	TT	3 (0.05)	0 (0.0)	7.659	0.384–152.642	3.17	0.07495
	C	99 (0.82)	108(0.87)	Ref			
	T	21 (0.18)	16 (0.13)	1.432	0.707–2.899	1.00	0.31691
rs3923086	TT	21 (0.36)	23 (0.37)	Ref			
	TG	30 (0.52)	29 (0.47)	1.133	0.519–2.475	0.10	0.75405
	GG	7 (0.12)	10 (0.16)	0.767	0.247–2.380	0.21	0.64528
	T	72 (0.62)	75 (0.60)	Ref			
	G	44 (0.38)	49 (0.40)	0.935	0.556–1.573	0.06	0.80113
rs3923087	AA	20 (0.33)	21 (0.34)	Ref			
	AG	24 (0.40)	26 (0.42)	0.969	0.424–2.215	0.01	0.94091
	GG	16 (0.27)	15 (0.24)	1.120	0.440–2.848	0.06	0.81187
	A	64 (0.53)	68 (0.55)	Ref			
	G	56(0.47)	56 (0.45)	1.062	0.642–1.758	0.06	0.81350
rs4791171	AA	18 (0.30)	21 (0.33)	Ref			
	AG	28 (0.47)	25 (0.40)	1.307	0.570–2.994	0.40	0.52679
	GG	14 (0.23)	16 (0.26)	1.021	0.393–2.651	0.00	0.96622
	A	64 (0.53)	67 (0.54)	Ref			
	G	56 (0.47)	57 (0.46)	1.029	0.622–1.701	0.01	0.91284
rs4135385	AA	47 (0.78)	41 (0.66)	Ref			
	AG	13 (0.22)	19 (0.31)	0.597	0.263–1.356	1.53	0.21550
	GG	0 (0.0)	2 (0.03)	0.175	0.008–3.745	2.24	0.13485
	A	107(0.89)	101(0.81)	Ref			
	G	13 (0.11)	23 (0.19)	0.534	0.256–1.110	2.89	0.08935
rs13072632	CC	28 (0.47)	30 (0.48)	Ref			
	CT	20 (0.34)	26 (0.42)	0.824	0.379–1.794	0.24	0.62595
	TT	11 (0.19)	6 (0.10)	1.964	0.641–6.021	1.42	0.23310
	C	76 (0.64)	86 (0.69)	Ref			
	T	42 (0.36)	38 (0.31)	1.251	0.731–2.139	0.67	0.41342
rs6485350	AA	21 (0.36)	18 (0.29)	Ref			
	AG	31 (0.53)	32 (0.52)	0.830	0.373–1.848	0.21	0.64873
	GG	7 (0.11)	12 (0.19)	0.500	0.162–1.540	1.48	0.22388
	A	73 (0.62)	68 (0.55)	Ref			
	G	45 (0.38)	56 (0.45)	0.749	0.448–1.250	1.23	0.26793
rs12255372	GG	25 (0.43)	26 (0.42)	Ref			
	GT	27 (0.47)	31 (0.50)	0.906	0.426–1.924	0.07	0.79687
	TT	6 (0.10)	5 (0.09)	1.248	0.338–4.614	0.11	0.73956
	G	77 (0.67)	83 (0.67)	Ref			
	T	39 (0.33)	41 (0.33)	1.025	0.599–1.754	0.01	0.92722

to demonstrate its role in colorectal carcinogenesis as well as other diseases.

Significant risk association of developing colorectal cancer was not observed with 12 of the 13 SNPs examined in AXIN2, APC, SFRP3, LRP6, DKK3, DKK4, and TCF4 as well as with one of the SNPs in β-catenin gene (rs13072632) in the overall study population (Table 2). Fernández-Rozadilla and colleagues examined a set of 37 SNPs in Wnt and BMP pathways different than those in our study except for rs459552 in APC and observed no association with colorectal cancer in Spanish population (Fernandez-Rozadilla et al., 2010). It may be conceived that individually these SNPs might be posing little or no risk and may be exerting its effect in combination with other genetic variants or factors. Alternatively, other SNPs in these genes or possibly other genes in the Wnt pathway may have a greater role to play in the initiation of colorectal cancers.

In the present study, we performed pathway based genetic association and identified three SNPs in critical genes in Wnt signaling to be significantly associated with reduced colorectal cancer risk. Genetic variants in SFRP3 (rs7775), β-catenin (rs4135385) and LRP6 (rs2284396) genes correlated with considerable protection against colorectal cancer in our population. Though, the sample size is small in our study, the findings are noteworthy that need to be validated in larger and ethnically diverse groups for the identified potential genetic markers to be used for colorectal cancer screening.

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