Association of Genetic Variation in Genes Implicated in the β-Catenin Destruction Complex with Risk of Breast Cancer

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Abstract

Aberrant Wnt/β-catenin signaling leading to nuclear accumulation of the oncogene product β-catenin is observed in a wide spectrum of human malignancies. The destruction complex in the Wnt/β-catenin pathway is critical for regulating the level of β-catenin in the cytoplasm and in the nucleus. Here, we report a comprehensive study of the contribution of genetic variation in six genes encoding the β-catenin destruction complex (*APC, AXIN1, AXIN2, CSNK1D, CSNK1E*, and *GSK3B*) to breast cancer using a Mayo Clinic Breast Cancer Case-Control Study. A total of 79 candidate functional and tagging single nucleotide polymorphisms (SNP) were genotyped in 798 invasive cases and 843 unaffected controls. Of these, rs454886 in the *APC* tumor suppressor gene was associated with increased breast cancer risk (per allele odds ratio, 1.23; 95% confidence intervals, 1.05-1.43; P_{trend} = 0.01). In addition, five SNPs in *AXIN2* were associated with increased risk of breast cancer (P_{trend} < 0.05). Haplotype-based tests identified significant associations between specific haplotypes in *APC* and *AXIN2* ($P \leq 0.03$) and breast cancer risk. Further characterization of the *APC* and *AXIN2* variants suggested that *AXIN2* rs4791171 was significantly associated with risk in premenopausal (P_{trend} = 0.0002) but not in postmenopausal women. The combination of our findings and numerous genetic and functional studies showing that APC and AXIN2 perform crucial tumor suppressor functions suggest that further investigation of the contribution of *AXIN2* and *APC* SNPs to breast cancer risk are needed. (Cancer Epidemiol Biomarkers Prev 2008;17(8):2101–8)

Introduction

Wnt/β-catenin signaling is an essential intracellular pathway in early embryonic development and multiple other physiologic processes. During development, it is involved in body axis formation. In adult tissues, it controls homeostatic self-renewal. Wnts are secreted glycoproteins that signal across the membrane by binding to transmembrane receptors of the Frizzled (Fz) family (FZD1 to FZD10 in humans) and the low-density lipoprotein–related protein family (LRP5 and LRP6; ref. 1). In the presence of Wnt, Wnt-Fz-LRP complexes at the cell surface induce stabilization and nuclear localization of β-catenin by compromising its destruction complex (APC complex) in the cytoplasm, which is composed mainly of adenomatosis polyposis coli (APC), casein kinase 1 (CK1), glycogen synthase kinase-3β (GSK3β), and scaffold proteins AXIN1 and AXIN2 (2). As a result, β-catenin accumulates in the nucleus and associates with T cell factor/lymphocyte enhancer factor to activate a number of target genes that promote cell growth, proliferation, and survival. In the absence of Wnt ligands, GSK3β and CK1 kinases phosphorylate β-catenin in the destruction complex leading to ubiquitination and proteosomal degradation.

In the multiprotein destruction complex, APC acts as a scaffold protein providing the binding sites for the AXIN1 scaffold protein and β-catenin (3, 4). GSK3β associates with the destruction complex through the binding site in AXIN1 and phosphorylates β-catenin that is subsequently targeted for proteosomal degradation (5, 6). *AXIN2*, the homologue of *AXIN1*, encodes a protein with 60% amino acid identity to AXIN1 and both proteins contain the same putative conserved domains for binding to APC, GSK3β, CK1, and β-catenin (3). Although tissue distribution and transcriptional regulation of these two genes are significantly different, the two proteins are functionally equivalent *in vivo* (7). CK1 kinase consists of several isoforms including CK1α, CK1δ, CK1ε, and CK1γ. CK1δ/ε positively regulates Wnt signaling by mediating the phosphorylation of the APC, AXIN1, and β-catenin, components of the destruction complex leading to dissociation of the complex and stabilization of β-catenin (8, 9). The function of CK1α in Wnt signaling is not yet clear and its role in secondary axis formation in *Xenopus* embryos is still controversial (8, 10). CK1γ is not directly bound to the destruction complex. It activates Wnt signaling via phosphorylation of LRP6 increasing its affinity for AXIN and recruiting the protein to the cell surface receptor away from the complex (11).

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Hyperactivity of the Wnt/β-catenin signaling cascade as a result of genetic changes in major components of the pathway contributes to numerous types of human cancers (12). Inactivating mutations have been detected in *APC, AXIN1*, and *AXIN2*, all of which encode the core of the destruction complex. Activating mutations in *APC* are the early and initiating events in >80% of the familial colorectal cancers (13). Somatic mutations in the *AXIN2* gene are also found in some colorectal cancer cases with defective mismatch repair and germ line *AXIN2* mutations seem to predispose to colorectal cancers (14, 15). Biallelic mutations in *AXIN1* have been identified in hepatocellular carcinomas. Critical serine/threonine residues at the NH_2 terminus of β-catenin that affect the stability of the protein are mutated in a wide variety of human cancers including colon, liver, kidney, ovary, and pancreas (12). Surprisingly, such genetic mutations have rarely been detected in either familial or sporadic breast cancers. However, the development of mammary tumors in Wnt1, Wnt3, and Wnt10b transgenic mice suggests a link between the Wnt pathway and breast cancer (16, 17).

Moreover, certain mouse strains carrying *Apc* truncating mutations show enhanced sensitivity to carcinogen-induced mammary tumors and transgenic mice expressing stabilized β-catenin in the mammary glands develop carcinomas (18). Furthermore, biochemical and immunohistochemical analysis of human breast cancer specimens has shown elevation of cytoplasmic and/or nuclear β-catenin and enhanced β-catenin transcriptional activity in >60% of the primary tumor tissues (19, 20). Thus, the Wnt/β-catenin pathway seems to play a significant role in the development and progression of human breast cancer. Given the role of the pathway in breast cancer and the absence of mutations in the destruction box genes, we conducted an association study to determine whether common genetic variations in six genes (*APC, AXIN1, AXIN2, CSNK1D, CSNK1E*, and *GSK3B*) which encode the destruction complex of the Wnt/β-catenin signaling pathway account in part for the contribution of the pathway to breast cancer risk.

Materials and Methods

Study Population. Details concerning the collection of cases and controls used in the Mayo Clinic Breast Cancer Case-Control Study have been described previously (21). Briefly, this population is a clinic-based six-state series of breast cancer cases and unaffected controls from Minnesota, Iowa, Wisconsin, North and South Dakota, and Illinois recruited from 2002 to 2005. All cancer patients were newly diagnosed with invasive breast cancer and had no prior history of cancer, other than nonmelanoma skin cancer. Controls with no history of cancer were recruited in parallel from women attending the Department of Internal Medicine at the Mayo Clinic for a prescheduled general medical exam. Controls were frequency-matched to cases by age, menopausal status, and state of residence. Eligible women provided informed consent and a sample of blood as a source of DNA and completed a risk factor questionnaire. Participation rates were 69% and 71% for cases and controls, respectively. Genomic DNA from 805 Caucasian invasive cases and 843 matched Caucasian controls were used in this study. Estrogen receptor (ER) status, progesterone receptor (PR) status, and human epidermal growth factor receptor 2 (HER2) status of tumors was available for 483 (60%), 437 (54%), and 127 (16%) of cases, respectively.

Single Nucleotide Polymorphism Selection. Single nucleotide polymorphisms (SNP) in the genomic region from 5 kb upstream to 5 kb downstream of each of the six destruction box genes with minor allele frequencies (MAF) of >0.05 in Caucasian populations were selected from the HapMap Consortium Stage II release (22). Tagging SNPs representing SNPs with pairwise correlation of $r^2 \geq 0.8$ were chosen by ldSelect. Preference was given to SNPs with a high probability of assay conversion on the Illumina Goldengate Platform (23). Functional SNPs were downloaded from the Ensembl web site[3] (NCBI35) and included synonymous and nonsynonymous coding SNPs, 5′- and 3′-untranslated region SNPs, and 5′ upstream (promoter region) SNPs within 1,000 bp of the transcriptional start site of each gene. From among the functional SNPs, only those with MAF > 0.05 in the Caucasian population were selected. A total of 70 tagSNPs and 9 functional SNPs were genotyped.

Biospecimen Processing and Genotyping. Blood samples obtained from participating cases and controls were processed by the Biospecimen Accessioning and Processing Shared Resource in the Mayo Clinic Cancer Center. Genomic DNA was resuspended in TE buffer at a uniform concentration of 0.25 μg/μL, and stored at −20°C. A total of 1,748 samples (805 cases, 843 controls, and 100 duplicates) were assayed on an Illumina BeadLab using the Illumina GoldenGate genotyping assay. Five cases were later found to have noninvasive tumors and were excluded, whereas DNA from two additional cases that failed genotyping were also omitted from analyses (798 cases included in statistical analysis).

Statistical Analysis. Departures from Hardy-Weinberg equilibrium for each SNP were examined using Pearson goodness-of-fit χ^2 statistics, or exact tests when MAF < 0.05. Pairwise linkage disequilibrium (LD) was estimated using r^2 and D-prime statistics and graphically displayed using the Haploview software (24). Unconditional logistic regression analysis was used to estimate odds ratios (OR) and 95% confidence intervals (95% CI) for risk of breast cancer associated with each SNP (25), while adjusting for the matching variables of age and state of residence, as well as other covariates. Primary tests of association between genotype and breast cancer status assumed a multiplicative (log-additive) structure for each SNP, equivalent to the Armitage test for trend in the absence of covariate adjustment. To aid in interpretability, ORs and 95% CIs were estimated for individuals with one and two copies of the minor allele relative to those with zero copies. In order to obtain a measure of the degree to which the findings reported here might be false-positive results, q values were obtained. A q value estimates the probability that a P value as small, or smaller, than the one observed is a false-positive. Therefore, it can be interpreted to reflect the probability that a test of significance is a false-positive result (26-28). Additional analyses assessed SNP associations with risk of breast cancer within subsets defined by menopausal

[3] http:/www.ensembl.org

status and by ER, PR, and HER2 status of tumors. Risk factors that were significantly associated ($P < 0.05$) with case-control status were included in all multivariate analyses. These included age, state of residence, age at menarche, oral contraceptive use, age at first childbirth, menopausal status, hormone replacement therapy, and pack-years of smoking.

Gene level associations were assessed between inferred haplotypes and breast cancer status using the score test implemented in haplo.stats comparing each haplotype to all others combined (29). In addition, haplotype blocks within each of the six genes of interest were defined using the method of Gabriel et al. (30) implemented in Haploview and associations between haplotypes in haplotypes blocks and risk were evaluated. All analyses were carried out using SAS (SAS Institute) and S-plus (Insightful Corporation) software.

Results

Individual SNPs Associated with Breast Cancer Risk. The characteristics of the Mayo Clinic Breast Cancer Case-Control Population used in this study have been previously reported (21). We selected a total of 79 SNPs from six genes encoding the core components of the β-catenin destruction complex (*APC, AXIN1, AXIN2, CSNK1D, CSNK1E*, and *GSK3B*; Table 1). Among these, 70 were tagSNPs and 9 were putatively functional SNPs (four 5′ upstream, three 3′-untranslated region, and two nonsynonymous SNPs) with MAF > 0.05. The coverage of the genetic variation in each gene by the tagSNPs, based on the HapMap database (NCBI Build 34), is shown in Table 1. Genotyping data for 71 of the 79 SNPs (19 *APC*, 21 *AXIN1*, 11 *AXIN2*, 2 *CSNK1D*, 7 *CSNK1E*, and 11 *GSK3B* SNPs) on 798 breast cancer cases and 843 controls was obtained and used for further statistical analysis. These SNPs displayed high call rates (>95%), MAF > 0.05 and did not diverge significantly from Hardy-Weinberg equilibrium (Supplementary Table S1).

SNPs in the *APC* and *AXIN2* genes were significantly associated with breast cancer risk ($P_{trend} < 0.05$; Table 2) under a log-additive model. These SNPs were estimated to have a probability of being true-positives of ~85% ($q = 0.15$). In the *APC* gene, the minor allele of rs454886 was associated with an increased risk of breast cancer (OR, 1.23; 95% CI, 1.05-1.43; $P_{trend} = 0.01$). The minor alleles of the other 10 *APC* SNPs shown in the table were significantly associated with decreased risk of breast cancer (OR, 0.77-0.85). Five SNPs from the *AXIN2* gene were significantly associated with an increased risk of breast cancer (OR, 1.18-1.28; Table 2). In addition to the primary analyses based on the log-additive model, we also evaluated the statistical significance of associations with one and two copies of these SNPs. ORs and P values for minor allele heterozygotes and homozygotes were similar to those observed under the log-additive model (Supplementary Table S2). No associations with breast cancer risk reached statistical significance for any of the SNPs in *AXIN1, CSNK1D, CSNK1E*, or *GSK3B*. All 16 SNPs from *APC* and *AXIN2* displaying significant associations with risk (Table 2) were noncoding tagSNPs (Fig. 1).

Haplotype Associations with Breast Cancer Risk. Gene-specific haplotype analyses were also done on all six genes to determine whether regions of genes and/or combinations of SNPs in genes were associated with breast cancer risk. Initially, we considered all *APC* SNPs together and applied the haplo.stat approach (31), using a combination of all other haplotypes with a frequency of >2% in the gene as the referent group. The *APC* (1111111211111111111) haplotype, which contains the minor allele of rs454886, was significantly associated with an increased risk of breast cancer (Hap-Score, 2.68; $P = 0.008$; Supplementary Table S3). Likewise, a specific haplotype containing all of the SNPs in *AXIN2* displayed a marginally significant association with risk (Hap-Score, 1.97; $P = 0.05$; Supplementary Table S3). However, because these combinations of SNPs do not reflect actual haplotypes, we further divided the genes into haplotype blocks ($r^2 \geq 0.8$) using Haploview, an LD-based partitioning algorithm (30), as shown in Fig. 1. Specific haplotypes in *APC* (blocks 1 and 2) and *AXIN2* (block 1) were significantly associated ($P < 0.05$) with risk (Table 3). In *APC* block 1, one haplotype (11111112) containing the minor allele of rs454886 was significantly associated with increased risk (Hap-Score, 2.73; $P = 0.006$). This was consistent with the observation that the minor allele of rs454886 was associated with an increased risk of disease (Table 2).

Interestingly, we also found that haplotype 11222221 in the same haplotype block was associated with decreased risk (Hap-Score, −2.16; $P = 0.03$). This conforms with the apparent protective effect of the minor alleles of several of these APC SNPs shown in Table 2. In *APC* block 2, the haplotype consisting of all

Table 1. SNPs genotyped for the core components of destruction complex genes in Wnt/β-catenin signaling pathway

Wnt genes	HapMap-CEU* no.		No. of genotyped SNP			TagSNP density
	SNP	Bin	Tagged	Functional	Total	SNP per kb
APC	90	13	13	7	20	0.10
AXIN1	38	23	23	2	25	0.27
AXIN2	15	13	13	0	13	0.26
CSNK1D	4	3	3	0	3	0.06
CSNK1E	8	7	7	0	7	0.15
GSK3B	99	11	11	0	11	0.04

NOTE: TagSNP density was calculated based on chromosome start and end points of the gene in NCBI Build 34 plus 5 kb upstream and 5 kb downstream regions.

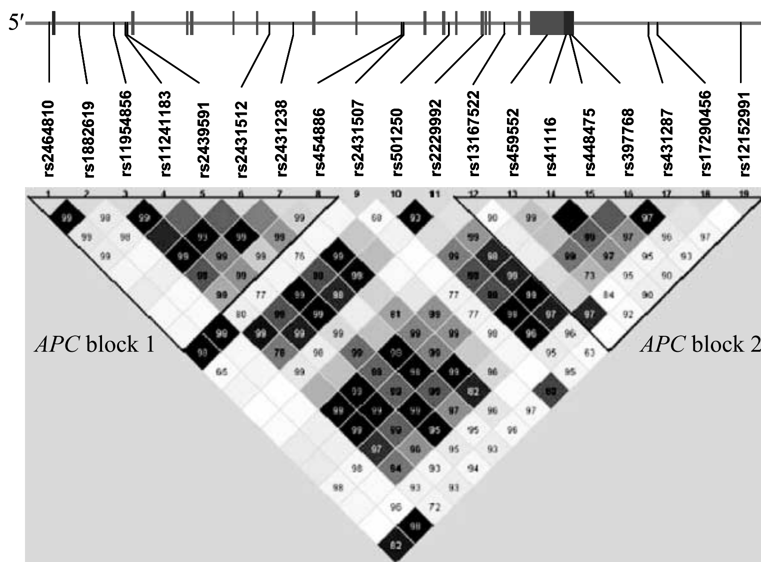
* HapMap-CEU, Utah HapMap samples (derived from United States residents with northern and western European ancestry). HapMap-CEU number includes those SNPs with MAF > 0.05.

Table 2. Multivariate analysis of polymorphic variants of *APC* and *AXIN2* genes

Gene name	rsID	Homozygotes, major allele		Heterozygotes		Homozygotes, minor allele		OR (95% CI) per allele	P_{trend}*
		Cases	Controls	Cases	Controls	Cases	Controls		
APC	rs11954856	261	229	386	430	150	181	0.84 (0.72-0.97)	0.02
APC	rs11241183	223	190	391	432	184	221	0.84 (0.73-0.97)	0.02
APC	rs2439591	366	333	339	394	93	115	0.85 (0.74-0.99)	0.04
APC	rs2431512	225	192	391	431	182	220	0.84 (0.73-0.97)	0.02
APC	rs2431238	400	360	317	387	81	95	0.85 (0.73-0.99)	0.04
APC	rs454886	358	422	346	355	94	66	1.23 (1.05-1.43)	0.01
APC	rs501250	346	318	352	397	99	127	0.85 (0.73-0.98)	0.03
APC	rs2229992	317	284	362	409	118	149	0.85 (0.74-0.99)	0.04
APC	rs13167522	675	677	117	157	6	9	0.77 (0.60-0.98)	0.04
APC	rs41116	224	192	390	431	184	220	0.85 (0.73-0.98)	0.02
APC	rs448475	224	191	390	430	184	221	0.84 (0.73-0.97)	0.02
AXIN2	rs7210356	626	677	154	159	18	6	1.28 (1.02-1.60)	0.04
AXIN2	rs4791171	383	433	332	349	83	61	1.19 (1.02-1.39)	0.03
AXIN2	rs11079571	533	606	233	221	32	16	1.28 (1.05-1.54)	0.01
AXIN2	rs3923087	458	525	292	278	47	39	1.19 (1.00-1.41)	0.04
AXIN2	rs3923086	238	284	395	419	164	139	1.18 (1.02-1.36)	0.03

NOTE: Logistic regression analysis was adjusted for age, geographic region, menopausal status, age at menarche, oral contraceptive use, age at first birth, hormone replacement therapy, and pack-years of cigarette smoking.
Abbreviation: rsID, SNP identification number.
*For these ordinal P values, the false-positive probability q = 0.15 except for rs3923087 (q = 0.16).

the major alleles (11111111) was associated with increased risk (Hap-Score, 2.33; P = 0.02), when using all other haplotypes of frequency >2% as a referent group (Table 3). To further explore this association, we evaluated the influence of the various APC block 2 haplotypes on risk using the wild-type (11111111) haplotype as the referent group (data not shown). In this setting, the haplotype (21222221) was significantly associated with a decreased risk (Hap-Score, 0.74; P = 0.05). This is consistent with the finding that the minor alleles of most of these individual SNPs in this haplotype block were associated with a reduced risk of cancer (Table 2). In *AXIN2* block 1, haplotype 11212 (Hap-Score, 2.20; P = 0.03) was associated with increased risk of breast cancer. This was the only haplotype that contained the minor allele of rs7210356, which was also significantly associated with increased risk, suggesting that the association between the haplotype and risk is in large part dependent on the individual SNP. The finding that variation in *APC* and *AXIN2* at the haplotype level was associated with breast cancer risk provided supporting evidence that single SNPs in APC and AXIN2



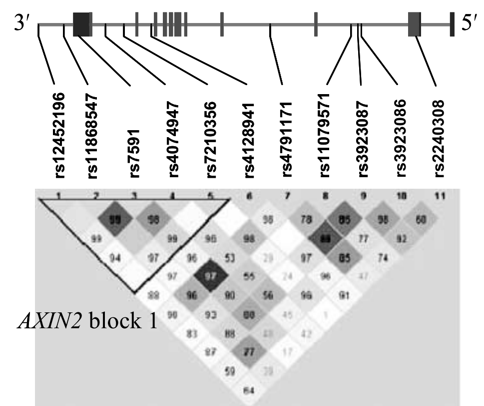


Figure 1. Pairwise LD for SNPs in the *APC* and *AXIN2* genes arrayed by physical location. Blocks of high LD are outlined as triangles and numbered as indicated in the figure. Shading reflects differences in pairwise LD (*white*, r^2 = low LD; *black*, r^2 = near-perfect LD). Numbers in squares are estimates of pairwise D-prime, expressed in percentages. Unreported values reflect D-prime of 1.0 (100%).

contributed to breast cancer risk and suggested that common haplotypes in *APC* also reduced breast cancer risk.

Effects of Menopausal Status and Tumor Pathology on Associations with Breast Cancer. In an effort to assess the influence of hormonal factors on these associations, data were stratified according to menopausal status (Table 4). The *APC* SNPs rs13167522 (P_{trend} = 0.02) and rs17290456 (P_{trend} = 0.02) displayed ~50% decreased risk and rs11241183, rs2431512, rs41116, and rs448475 exhibited ~25% decreased risk in premenopausal women, whereas the *APC* rs2439591, rs2431238, rs501250, and rs2229992 SNPs showed significantly decreased risk in postmenopausal women. All of these SNPs, except for rs17290456, displayed similar associations with breast cancer in the overall case-control study. None of the other APC SNPs previously associated with risk in this study population reached statistical significance in either menopausal stratum. In particular, the rs454886 SNP that was significantly associated with increased risk in the overall study failed to reach significance in the strata. This may result from reduced statistical power in the stratified analyses because the SNP displayed a positive association with risk in both groups. For *AXIN2*, four out of five SNPs previously associated with risk in the overall population, along with rs7591 that previously failed to reach significance, were significantly associated with increased risk in premenopausal but not in postmenopausal women (Table 4). Notably, the strength and the significance of the association between *AXIN2* rs4791171 and risk was substantially enhanced (OR, 1.84; 95% CI, 1.34-2.53; P_{trend} = 0.0002) among premenopausal cases and controls.

We also conducted an exploratory analysis of the relationship between the *APC* and *AXIN2* SNPs and tumor histology. Specifically, we evaluated associations with risk based on ER, PR, and HER2/neu status of tumors. We did not conduct analyses for the ER− and PR− subgroups because of limited sample size. Interestingly, the *APC* rs454886 that was significantly associated with risk in the overall study population was also significantly associated with risk in the ER+ group (OR, 1.20; 95% CI, 1.00-1.44; P_{trend} = 0.04) but not in the PR+ and HER2+ groups (Table 5). None of the other *APC* SNPs displayed significant associations with risk of breast cancer in the stratified analyses. The *AXIN2* rs7210356 SNP was significantly associated with risk in ER+ (OR, 1.34; 95% CI, 1.03-1.73; P_{trend} = 0.03) and PR+ (OR, 1.38; 95% CI, 1.06-1.80; P_{trend} = 0.02) categories but not in the HER2+ group, whereas the *AXIN2* rs11079571 displayed significance in all three categories (Table 5). None of the other *AXIN2* SNPs maintained significant associations with breast cancer in the subgroup analyses.

Discussion

APC and AXIN2 are important tumor suppressors that function in the Wnt signaling pathway by regulating the level of β-catenin. APC and AXIN2 are essential interacting components of the β-catenin destruction complex that function to down-regulate β-catenin through feedback inhibition (32, 33). Although germ line and somatic *APC* mutations are infrequent in human primary breast tumors (34, 35), the predisposition of the ApcMin mouse model provides strong evidence in support of a role for APC in breast cancer (18). Specifically, the Apc-deficient murine mammary glands develop metaplasia and rapidly progress to neoplasia in the presence of p53 mutations (36). Similarly, Axin2 knockout mice are more prone to carcinogen-induced neoplasia. In addition, epithelial-to-mesenchymal transition in invasive human breast cancers is associated with an AXIN2-dependent pathway

Table 3. Association of *APC* and *AXIN2* haplotypes with breast cancer risk

Haplotype blocks	SNP combinations*	Hap-frequency†	Hap-score‡	Haplotype P§
APC block 1	11222221	0.319	−2.16	0.03
	22221211	0.084	−0.53	0.60
	11222211	0.029	−0.07	0.95
	11121211	0.042	−0.06	0.95
	11111111	0.193	−0.02	0.98
	11111112	0.310	2.73	0.006
APC block 2	21222221	0.063	−1.56	0.12
	21222211	0.028	−1.10	0.27
	11221112	0.097	−0.75	0.45
	12222211	0.175	−0.66	0.51
	12222111	0.041	−0.65	0.51
	11221111	0.044	−0.08	0.94
	11222211	0.043	0.68	0.49
	11111111	0.500	2.33	0.02
AXIN2 block 1	12111	0.464	−0.93	0.35
	21111	0.141	−0.68	0.49
	11211	0.069	−0.47	0.64
	11221	0.210	0.28	0.78
	11212	0.108	2.20	0.03

NOTE: Haplotypes with frequency > 2% are shown in the table and those with P < 0.05 are underlined. *APC* block 1: rs2464810, rs1882619, rs11954856, rs11241183, rs2439591, rs2431512, rs2431238, and rs454886; *APC* block 2: rs13167522, rs459552, rs41116, rs448475, rs397768, rs431287, rs17290456, and rs12152991; *AXIN2* block 1: rs12452196, rs11868547, rs7591, rs4074947, and rs7210356.

*1, major allele; 2, minor allele.

†Frequency of each haplotype in the cases and controls.

‡Statistical measurement of association of each specific haplotype with breast cancer risk.

§Haplotype of interest compared with all other haplotypes (frequency >2%) combined.

Table 4. Association of *APC* and *AXIN2* SNPs with breast cancer risk in premenopausal and postmenopausal women

Gene name	rsID	Premenopausal women (N_{ca} = 273, N_{co} = 220)			Postmenopausal women (N_{ca} = 480, N_{co} = 579)		
		OR (95% CI) per allele	P_{trend}	*q* value	OR (95% CI) per allele	P_{trend}	*q* value
APC	rs11954856	0.79 (0.60-1.03)	0.09	0.24	0.84 (0.70-1.01)	0.06	0.20
APC	rs11241183	0.75 (0.57-0.98)	0.04	0.17	0.86 (0.72-1.03)	0.09	0.24
APC	rs2439591	0.90 (0.68-1.19)	0.44	0.45	0.80 (0.67-0.97)	0.02	0.17
APC	rs2431512	0.74 (0.56-0.97)	0.03	0.17	0.86 (0.72-1.03)	0.10	0.24
APC	rs2431238	0.87 (0.65-1.16)	0.34	0.44	0.81 (0.67-0.98)	0.03	0.17
APC	rs454886	1.26 (0.95-1.69)	0.11	0.25	1.19 (0.98-1.45)	0.07	0.22
APC	rs501250	0.87 (0.66-1.15)	0.33	0.44	0.81 (0.67-0.97)	0.03	0.17
APC	rs2229992	0.85 (0.65-1.12)	0.26	0.40	0.82 (0.69-0.99)	0.04	0.17
APC	rs13167522	0.57 (0.36-0.91)	0.02	0.17	0.86 (0.63-1.17)	0.33	0.44
APC	rs41116	0.76 (0.58-1.00)	0.05	0.19	0.86 (0.72-1.03)	0.11	0.25
APC	rs448475	0.75 (0.57-0.98)	0.04	0.17	0.86 (0.72-1.03)	0.10	0.24
*APC**	rs17290456	0.52 (0.30-0.88)	0.02	0.17	0.85 (0.59-1.24)	0.40	0.44
*AXIN2**	rs7591	1.46 (1.08-1.96)	0.01	0.17	1.03 (0.85-1.23)	0.79	0.56
AXIN2	rs7210356	1.53 (0.98-2.40)	0.06	0.20	1.19 (0.90-1.57)	0.22	0.35
AXIN2	rs4791171	1.84 (1.34-2.53)	0.0002	0.02	1.01 (0.84-1.23)	0.88	0.59
AXIN2	rs11079571	1.78 (1.21-2.63)	0.004	0.16	1.10 (0.87-1.39)	0.43	0.44
AXIN2	rs3923087	1.62 (1.14-2.28)	0.007	0.17	1.03 (0.83-1.27)	0.79	0.56
AXIN2	rs3923086	1.44 (1.08-1.93)	0.01	0.17	1.08 (0.90-1.29)	0.42	0.44

NOTE: Logistic regression analysis was adjusted for age, geographic region, age at menarche, oral contraceptive use, age at first birth, hormone replacement therapy, and pack-years of cigarette smoking.
Abbreviations: rsID: SNP identification number; N_{ca}, number of cases; N_{co}, number of controls.
*Nonsignificant association in complete case-control study population.

that stabilizes β-catenin and GSK3β and regulates SNAIL1 activity (37). These observations led to the hypothesis that genetic variation influencing the expression and/or function of the APC and AXIN2 tumor suppressors influences breast cancer risk.

In this study, we successfully genotyped 71 SNPs from six destruction complex genes of the canonical Wnt/β-catenin signaling pathway in 798 invasive breast cancer cases and 843 unaffected controls collected from the Mayo Clinic. We found that five *AXIN2* SNPs displayed significant associations with an increased risk of breast cancer as individual SNPs and as a common haplotype. Several of these SNPs were in high LD in the Mayo Clinic study population, as shown in Fig. 1, raising the possibility that a single variant in *AXIN2* might account for this effect. Haplotype analysis suggested that rs7210356 was responsible for the association with breast cancer. We also found that the *APC* SNP rs454886 was significantly associated with an increased risk of breast cancer. However, 10 other *APC* SNPs were individually associated with a decreased risk of breast cancer. Further analysis showed that a common haplotype containing the minor allele of only the rs454886 SNP was significantly associated with increased risk of breast

Table 5. Association of SNPs in *APC* and *AXIN2* with breast cancer by ER, PR, and HER2 status of tumors

Gene name	rsID	ER positive (N_{ca} = 483, N_{co} = 843)		PR positive (N_{ca} = 437, N_{co} = 843)		HER2 positive (N_{ca} = 127, N_{co} = 843)	
		OR (95% CI) per allele	P_{trend}*	OR (95% CI) per allele	P_{trend}*	OR (95% CI) per allele	P_{trend}*
APC	rs11954856	0.85 (0.72-1.01)	0.06	0.89 (0.74-1.06)	0.18	0.89 (0.67-1.18)	0.43
APC	rs11241183	0.87 (0.74-1.03)	0.11	0.91 (0.77-1.09)	0.31	0.91 (0.69-1.20)	0.51
APC	rs2439591	0.87 (0.73-1.03)	0.10	0.91 (0.76-1.09)	0.30	1.03 (0.78-1.37)	0.83
APC	rs2431512	0.86 (0.73-1.02)	0.08	0.90 (0.76-1.07)	0.23	0.92 (0.69-1.21)	0.55
APC	rs2431238	0.84 (0.70-1.00)	0.06	0.88 (0.74-1.06)	0.19	0.98 (0.73-1.32)	0.89
APC	rs454886	1.20 (1.00-1.44)	0.04	1.16 (0.96-1.39)	0.13	1.16 (0.86-1.56)	0.34
APC	rs501250	0.86 (0.72-1.02)	0.08	0.90 (0.75-1.07)	0.24	1.03 (0.77-1.36)	0.86
APC	rs2229992	0.88 (0.75-1.04)	0.15	0.93 (0.78-1.10)	0.40	1.00 (0.76-1.32)	0.99
APC	rs13167522	0.81 (0.61-1.08)	0.15	0.90 (0.67-1.20)	0.47	0.92 (0.57-1.46)	0.71
APC	rs41116	0.87 (0.74-1.03)	0.11	0.91 (0.77-1.09)	0.31	0.89 (0.68-1.18)	0.42
APC	rs448475	0.87 (0.73-1.03)	0.10	0.91 (0.76-1.08)	0.28	0.88 (0.67-1.17)	0.39
AXIN2	rs7210356	1.34 (1.03-1.73)	0.03	1.38 (1.06-1.80)	0.02	1.10 (0.69-1.73)	0.69
AXIN2	rs4791171	1.14 (0.95-1.36)	0.17	1.15 (0.95-1.39)	0.14	1.25 (0.92-1.69)	0.15
AXIN2	rs11079571	1.25 (1.01-1.56)	0.04	1.30 (1.04-1.63)	0.02	1.44 (1.00-2.06)	0.05
AXIN2	rs3923087	1.10 (0.90-1.34)	0.37	1.12 (0.92-1.38)	0.26	1.30 (0.95-1.79)	0.10
AXIN2	rs3923086	1.07 (0.91-1.27)	0.40	1.10 (0.93-1.31)	0.27	1.13 (0.85-1.50)	0.39

NOTE: Logistic regression analysis was adjusted for age, geographic region, menopausal status, age at menarche, oral contraceptive use, age at first birth, hormone replacement therapy, and cigarette smoking in pack-years.
Abbreviations: rsID: SNP identification number; N_{ca}, number of cases; N_{co}, number of controls.
*The false-positive probability *q* values were not calculated.

cancer, whereas two independent haplotypes containing the minor alleles of several of the other SNPs in *APC* were protective. These data suggest the existence of SNPs and haplotypes within *APC* that have opposite effects on breast cancer risk.

To better understand the relationship between these genes and breast cancer risk, we examined *APC* and *AXIN2* SNPs in premenopausal and postmenopausal subgroups of the Mayo Clinic Breast Cancer Study. Importantly, we observed a substantial increase in the significance of the association for several *AXIN2* SNPs in premenopausal women. Five of 13 *AXIN2* SNPs displayed significant associations with breast cancer in the premenopausal population whereas no significant associations were observed when restricting to postmenopausal women. Strikingly, rs4791171 displayed a 100-fold enrichment in the significance of the association with risk (P_{trend} = 0.0002 versus 0.03) and maintained significance even when accounting for multiple testing by Bonferroni correction (P_{trend} = 0.01). These data strongly suggest that genetic variation in *AXIN2* contributes to breast cancer in premenopausal women. Further studies will be needed to better define the contribution of age and hormonal exposure to this effect.

Ten *APC* SNPs again showed significance when restricting to premenopausal and postmenopausal populations. A series of SNPs in high LD in the 3′ end of the gene (rs13167522-rs17290456) seemed to be more protective in premenopausal women than in the overall population and were not significantly associated with breast cancer in postmenopausal women. In contrast, a series of SNPs in high LD in the 5′ end of the gene were significantly associated with a protective effect in the postmenopausal population. In addition, although nonsignificant, the rs4548867 SNP displayed the same increased risk of breast cancer in both subgroups. When considering these data in conjunction with the haplotype results, the suggestion is that the *APC* gene has two independent regions/haplotypes that decreases the risk of breast cancer and a single SNP in limited LD with the other tagSNPs that increases risk. Interestingly, none of these effects were observed when stratifying by the ER, PR, and HER2 status of tumors.

A number of studies have previously evaluated the contribution of genetic variation in *APC* and *AXIN2* to breast cancer. Trevor et al. reported that the *I1307K* nonsynonymous SNP in *APC* conferred a modestly elevated breast cancer risk in Ashkenazi Jewish Caucasians (38). However, the effect was not observed in non-Ashkenazi Caucasians because of the rarity of the *I1307K* allele. Furthermore, results from follow-up studies in Ashkenazi Jewish women with breast/ovarian cancer were contrary to the initial report (39, 40). In a separate association study of 640 SNPs in 111 genes including 7 *APC* SNPs in a Spanish breast cancer case-control population, no association with breast cancer risk for the *APC* SNPs was observed (41). However, because these seven SNPs do not account for the known genetic variation within *APC* (we selected 13 tagSNPs in the *APC* gene based on genotyping data for the Caucasian population from HapMap), it remains possible that associations with breast cancer were overlooked. In addition, population heterogeneity associated with differences in the Spanish and Mayo Clinic populations may also account for the absence of an observed significant association ($P < 0.05$). SNPs in *APC* and *AXIN2* have also been evaluated for associations with breast cancer risk in the Cancer Genetic Markers of Susceptibility (CGEMS) genome-wide association study. None of the associations with *APC* and *AXIN2* SNPs observed in our clinic-based case-control series were replicated in stage I of the CGEMS study, although the OR for rs454886 was in the same direction (CGEMS heterozygote risk OR, 1.18; P = 0.15). However, this result may be due to intrinsic heterogeneity between these two populations because the cohort used in the CGEMS study was primarily of postmenopausal women with invasive cancer (42). This is particularly relevant to SNPs in *AXIN2* which we found to be most significantly associated with risk in premenopausal women. While our report provides the first evidence to link the Wnt/β-catenin signaling pathway genes and their SNPs to breast cancer risk, the discrepancies between our results and CGEMS data further underscore the need for independent replication of our findings.

We took a comprehensive and systematic approach to this study. First, the genes selected for the study encoded protein components that form a well-characterized complex that is directly implicated in many cancers. Second, a homogeneous population restricted to a six-state demographic region around the Mayo Clinic was used to minimize potential population stratification effect, and third, the number of case and control subjects genotyped ensured that the study had sufficient statistical power to identify moderate to weak associations with risk. Moreover, genotyping of tagSNPs in combination with functional SNPs (MAF > 0.05) ensured a detailed and comprehensive coverage of genetic variation in the genes. However, calculation of the probability of false-positive results for each analysis in the form of q values (Tables 2 and 4) suggested that few of the observed associations survive adjustment for multiple testing. Thus, we recognize that additional studies which focus on the replication of our findings in independent case-control series, particularly in premenopausal women, with more detailed coverage of variation within these genes, are needed to establish the relevance of the observed associations to breast cancer.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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