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Genetic variants in frizzled-related protein (FRZB) and the risk of colorectal neoplasia

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Abstract

Objective—The Wnt/APC/ β -catenin signaling pathway, which includes frizzled-related protein (FRZB), plays a critical role in the development of colorectal cancer, and recent evidence suggests that the functional polymorphism, *FRZB Arg324Gly*, may be associated with risk for this disease. To determine if this finding could be replicated, we investigated the association between two *FRZB* polymorphisms (*Arg324Gly* and *Arg200Trp*) and the risk of colorectal adenoma and cancer in nested case-control studies.

Methods—Participants consisted of 1709 adenoma cases, 620 cancer cases, and 1849 controls within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial. Logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (95% CI) for the associations with colorectal neoplasia.

Results—No association was observed for either polymorphism or any haplotypes with colorectal adenoma or colorectal cancer ($P > 0.05$ for all).

Conclusion—Our study does not support the previously observed association between the *FRZB 324Gly* variant and colorectal cancer risk. However, further study of additional genetic variants within this pathway is still warranted, given the important role of the Wnt signaling pathway in colorectal carcinogenesis.

Keywords

Colorectal adenoma; colorectal cancer; polymorphism; Wnt signaling pathway

Introduction

The Wnt/APC/ β -catenin signaling pathway plays a central role in colorectal carcinogenesis with somatic alterations in *APC* leading to increased β -catenin levels and cell proliferation in many tumors (1). Germline mutations in *APC* bring about the inherited colorectal cancer syndrome, familial adenomatous polyposis (2). The frizzled-related protein, FRZB (also called secreted frizzled-related protein 3 or FRP3), impedes Wnt signaling, which regulates the accumulation of β -catenin levels in the cytoplasm. The *FRZB 324Gly* variant and the *200Trp/324Gly* double-variant haplotype have reduced ability to antagonize Wnt signaling leading to

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increased β -catenin levels (3), and a recent study reported that *FRZB* 324Gly homozygotes were associated with an increased risk of colorectal cancer (4). We evaluated the association between the *FRZB* Arg200Trp and Arg324Gly polymorphisms and the risk of colorectal neoplasia in a nested case-control study within the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial.

Materials and Methods

Study Population

The PLCO Cancer Screening Trial is a randomized trial to evaluate screening methods for prostate, lung, colorectal, and ovarian cancer (5). Participants randomized to the screening arm undergo sigmoidoscopy screening at baseline and years 3 or 5 (T3/T5). Participants found to have a suspicious lesion were referred to their primary physicians for evaluation, and diagnostic information was abstracted from the medical records by trained personnel, identifying pathologically verified cases of colorectal adenoma and cancer (6). Colorectal cancers were also identified through annual questionnaires and death certificates; all colorectal cancers were pathologically verified. The study was approved by the institutional review boards at the screening centers and the National Cancer Institute, NIH.

As described elsewhere (7), cases for the first adenoma study (PLCO I) were 1,463 participants from the screening arm with advanced adenoma (≥ 1 cm in size, containing villous/tubulovillous characteristics, high-grade dysplasia or carcinoma *in situ*) located in the distal colon or rectum identified at the baseline. Controls consisted of 1,631 participants who had a successful baseline screening exam that was negative for polyps in the distal colon and rectum, matched on ethnicity, sex, and for a subset, age. For the second adenoma study (PLCO II), cases included 407 participants who screened negative on the baseline exam but were found to have adenoma of the distal colon or rectum (≥ 0.5 cm in size, displaying villous/tubulovillous characteristics high-grade dysplasia or carcinoma *in situ*) on the T3/T5 exam. Controls were 407 subjects negative for adenomatous polyps of the distal colon and rectum on both the baseline and T3/T5 sigmoidoscopic exams and matched on ethnicity, sex, and age. Subjects for the colorectal cancer study included 651 confirmed colorectal cancer cases (355 cases from the screening arm and 296 from the control arm) and all controls from PLCO I and II.

Genotyping

DNA was available for 1709 colorectal adenoma cases, 620 colorectal cancer cases, and 1849 controls. Two single nucleotide polymorphisms (SNPs) in *FRZB* (rs288326, Arg200Trp; rs7775, Arg324Gly) were genotyped using TaqMan assays at the NCI Core Genotyping Facility (8). After stratification by ethnicity, the genotype frequencies among controls were consistent with Hardy-Weinberg proportions ($P > 0.10$) and quality control duplicate samples yielded 100% concordance.

Statistical methods

Logistic regression was used to assess the association between the *FRZB* variants and colorectal neoplasia, adjusting for age, sex, race, and for the pooled analysis, study (PLCO I vs. PLCO II). Although the minor allele frequencies differed by race (Arg200Trp: 0.12, 0.04, and 0.02; Arg324Gly: 0.08, 0.32, and 0.03 for Caucasians, African-Americans, and other races, respectively), the p-values for heterogeneity were not statistically significant in the logistic regression models (Arg200Trp: $P_{\text{heterogeneity}} = 0.22$ and 0.80; Arg324Gly: $P_{\text{heterogeneity}} = 0.44$ and 0.83 for adenoma and cancer, respectively). Thus, all races were included in the primary SNP analyses. Interactions with the SNPs were tested by comparing the logistic regression models with the main effect terms with and without the cross product term using a likelihood ratio test. Haplotypes were estimated among Caucasians only using an expectation

maximization (EM) algorithm and associations evaluated using the generalized linear model and global score test in HaploStats (9). Unless otherwise specified, analyses were conducted using SAS 9.1 (Cary, NC).

Results

We found no association between the *FRZB* polymorphisms, *Arg200Trp* and *Arg324Gly*, and colorectal adenoma or cancer (Table 1). The associations were similar when the analysis was restricted to Caucasians for both adenoma (OR = 0.98, 95% CI: 0.51-1.89 and OR = 1.00, 95% CI: 0.42-2.37 for the variant homozygotes at *Arg200Trp* and *Arg324Gly*, respectively) and cancer (OR = 1.60, 95% CI: 0.68-3.76 and OR = 1.05, 95% CI: 0.28-3.92 for the variant homozygotes at *Arg200Trp* and *Arg324Gly*, respectively). The polymorphisms were in weak linkage disequilibrium among Caucasians ($D' = 0.08$, $r^2 = 0.004$), and mutual adjustment had a negligible effect on the odds ratios. The polymorphisms were not associated with advanced adenoma (OR = 0.78, 95% CI: 0.38-1.59 and OR = 1.09, 95% CI: 0.53-2.25 for the variant homozygotes at *Arg200Trp* and *Arg324Gly*, respectively). No associations were observed with any of the haplotypes (Table 2).

There were no statistically significant differences in the SNP associations by tumor location for adenoma (distal vs. rectal) or cancer (colon vs. rectal, proximal vs. distal) ($P_{\text{heterogeneity}} > 0.05$); however, a borderline protective association was observed for carriers of the *T* allele at *Arg200Trp* compared to *CC* homozygotes for exclusively rectal adenoma (OR = 0.69, 95% CI: 0.49-0.99), which was attenuated and not statistically significant for the less restrictive outcome of at least one rectal adenoma (Supplementary Table 1) or rectal cancer (Supplementary Table 2). Although statistically significant interactions were found with *Arg200Trp* and smoking ($P = 0.02$), *Arg324Gly* and body mass index ($P = 0.03$), and *Arg324Gly* and aspirin/ibuprofen use ($P = 0.01$) for colorectal cancer (Supplementary Table 3), none of these interactions were observed with adenoma ($P > 0.05$), suggesting that these were likely to be spurious associations for the smaller cancer series. No significant interactions were found with age, sex, or family history for either variant for adenoma or cancer ($P > 0.05$).

Discussion

Although the *FRZB* 324Gly variant and *FRZB* 200Trp/324Gly double-substitution haplotype have been shown to have decreased ability to antagonize Wnt signaling *in vitro* (3), this study showed no association between the *FRZB* *Arg324Gly* or *Arg200Trp* polymorphisms or haplotypes and the risk of colorectal cancer or adenoma overall. In a previously published study with a similar number of cancer cases ($N = 659$) but smaller number of controls ($N = 607$), Shanmugam *et al.* found that *FRZB* 324Gly homozygotes had a five-fold increased risk of colorectal cancer (OR = 5.01, 95% CI: 1.71-14.63) (4). Our study did not replicate this result (OR = 1.09, 95% CI: 0.44-2.72). Shanmugam *et al.* also reported that the association was stronger for rectal cancer. We did not observe a positive association with rectal cancer in our study (OR = 0.62, 95% CI: 0.08-4.91); however, the number of rectal cancer cases in our study was small ($n = 152$) and only one case was homozygous for the *FRZB* 324Gly variant, making inferences difficult. It is possible that differences in environmental factors between the study populations contributed to the heterogeneity in results observed. We did observe statistically significant interactions between the *FRZB* *Arg324Gly* polymorphism and body mass index and aspirin/ibuprofen use; however, our sample size for colorectal cancer was small and we did not observe the similar interactions with colorectal adenoma.

In our study, we observed a borderline protective association for exclusively rectal adenoma with carriers of the *T* allele at *Arg200Trp*, but the association was slightly weaker and not statistically significant for the less restrictive outcome of at least one rectal adenoma and rectal

cancer. No association was observed between the *T* allele at *Arg200Trp* and rectal cancer in a study conducted in Germany (OR = 0.98, 95% CI: 0.69-1.38) (4). Thus, this subgroup finding should be interpreted with caution. Consistent with our study, Shanmugam et al. found no association with the *FRZB Arg200Trp/Arg324Gly* haplotypes.

Our study had several strengths and limitations. The large sample of colorectal adenoma and cancer cases allowed us to comprehensively evaluate the risk associated with a spectrum of colorectal neoplastic tumors, and we evaluated genetic variants that had been shown to have a functional effect on Wnt signaling. However, our study had limited power to detect a recessive genetic association, and it is possible that the positive association observed by Shanmugam et al. failed to replicate in our study due to the modest sample size for colorectal cancer and the fact that associations for adenoma may be different from those for cancer. Genetic variants associated with progression from adenoma to cancer or with colorectal cancers that arise *de novo* may not be associated with adenoma risk. In addition, our coverage of *FRZB* was not comprehensive. There may be other genetic variants within this genetic region that are related to risk which were not captured in our study.

Although we did not observe an association with the *FRZB Arg200Trp* or *Arg324Gly* polymorphisms and the risk of colorectal neoplasia, it is possible that other variants within *FRZB* or other genes within the Wnt signaling pathway may be associated with risk. Additional studies are needed to evaluate associations within this important pathway in colorectal carcinogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

(OR), Odds ratio; (95% CI), 95% confidence interval; (SNP), single nucleotide polymorphism; (PLCO), Prostate, Lung, Colorectal and Ovarian Cancer Screening Trial.

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Table 1
Risks of colorectal neoplasia associated with *FKZB* polymorphisms in the PLCO Cancer Screening Trial

SNP	Colorectal Adenoma						Colorectal Cancer		
	PLCO I*			PLCO II*			Pooled		
	Cases/Controls	OR* (95% CI)		Cases/Controls	OR* (95% CI)		Cases/Controls	OR* (95% CI)	OR* (95% CI)
rs288326 (<i>Arg200Trp</i>)									
CC	1044 / 1115	1.0		318 / 327	1.0		1362 / 1442	1.0	1.0
CT	247 / 309	0.85 (0.70-1.02)		80 / 75	1.10 (0.77-1.57)		327 / 384	0.90 (0.76-1.06)	1.04 (0.82-1.32)
TT	13 / 16	0.85 (0.41-1.78)		5 / 3	1.70 (0.40-7.17)		18 / 19	0.99 (0.52-1.90)	1.76 (0.78-4.01)
CT or TT									
<i>P</i> _{trend}		0.09			0.45			0.24	0.39
rs7775 (<i>Arg324Gly</i>)									
CC	1080 / 1187	1.0		337 / 341	1.0		1417 / 1528	1.0	1.0
CG	206 / 238	0.97 (0.78-1.19)		65 / 60	1.09 (0.74-1.60)		271 / 298	0.99 (0.82-1.19)	0.88 (0.67-1.14)
GG	13 / 13	1.18 (0.54-2.60)		1 / 4	0.25 (0.03-2.26)		14 / 17	0.94 (0.46-1.94)	1.09 (0.44-2.72)
CG or GG									
<i>P</i> _{trend}		0.90			0.93			0.86	0.44

‡ Odds ratios are adjusted for age, sex, race, and study (for the pooled adenoma analysis)

* PLCO I includes prevalent advanced adenoma cases detected on the baseline sigmoidoscopy examination and matched controls. PLCO II includes incident adenoma cases detected on the T3 or T5 sigmoidoscopy examination and matched controls.

Table 2
Risks of colorectal neoplasia associated with *FRZB* haplotypes among Caucasians in the PLCO Cancer Screening Trial

Haplotype [†]	Colorectal Adenoma						Colorectal Cancer		
	PLCO I*		PLCO II*		Pooled		Freq Cases/Controls	OR [‡] (95% CI)	OR [‡] (95% CI)
	Freq Cases/Controls	OR [‡] (95% CI)	Freq Cases/Controls	OR [‡] (95% CI)	Freq Cases/Controls	OR [‡] (95% CI)			
C C	0.82 / 0.81	1.0	0.81 / 0.83	1.0	0.82 / 0.81	1.0	0.81 / 0.81		1.0
C G	0.07 / 0.07	1.07 (0.85-1.35)	0.07 / 0.06	1.14 (0.74-1.75)	0.07 / 0.06	1.08 (0.89-1.33)	0.06 / 0.07		0.88 (0.64-1.21)
T C	0.10 / 0.11	0.86 (0.71-1.04)	0.11 / 0.09	1.23 (0.85-1.80)	0.10 / 0.11	0.93 (0.78-1.10)	0.11 / 0.11		1.08 (0.85-1.37)
T G	0.01 / 0.01	0.69 (0.35-1.33)	0.02 / 0.02	0.78 (0.30-2.02)	0.01 / 0.02	0.72 (0.42-1.24)	0.02 / 0.02		1.12 (0.59-2.15)
P _{global score test}		0.20		0.70		0.38			0.75

* PLCO I includes prevalent advanced adenoma cases detected on the baseline sigmoidoscopy examination and matched controls. PLCO II includes incident adenoma cases detected on the T3 or T5 sigmoidoscopy examination and matched controls.

[†] Haplotypes are comprised of the *Arg200Trp* (rs288326) and *Arg324Gly* (rs7775) genotypes, respectively

[‡] Odds ratios are adjusted for age, sex, race, and study (for the pooled adenoma analysis)