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Polymorphisms in WNT6 and WNT10A and Colorectal Adenoma Risk

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Abstract

The WNT/β-catenin signaling pathway upregulates transcription of genes involved in cell proliferation and cancer progression; it has been implicated in colorectal adenoma formation. To date, no studies have examined polymorphisms in WNT genes or WNT gene–environment interactions in relation to adenoma risk. Within a colonoscopy-based case-control study of 628 adenoma cases and 516 polyp-free controls, we analyzed two tagSNPs in WNT6 (rs6747776 G > C, rs6754599 G > C) and WNT10A (rs7349332 G > A, rs10177996 A > G). The WNT6

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rs6747776 homozygous minor allele (CC) was associated with increased risk of colorectal adenoma (OR = 2.75, 95% CI: 1.03–7.31). We observed a statistically significant interaction between WNT6 rs6747776 and the proportion of calories from total fat (*P*-int = 0.02), where the highest risk was observed among those with minor alleles and lowest fat intake. We also detected a marginally significant ($0.05 < P \leq 0.10$) interaction with fish intake (*P*-int = 0.09). Additionally, a marginally significant interaction was observed between proportion of calories from saturated fat and the WNT10A rs7349332 polymorphism. Our results suggest that genetic variability in the WNT pathway may play a role in colorectal adenoma formation or may partly mediate the increased risk of colorectal cancer associated with fat intake.

INTRODUCTION

WNT proteins are members of a family of secreted, cysteine-rich proteins involved in multiple cellular activities (1,2). Signaling through the WNT/β-catenin pathway results in the transcription of several genes important for carcinogenesis, including MMP7, which is involved in tumor invasion and metastasis; antiapoptotic proteins such as caspase inhibitors and survivin; vascular endothelial growth factor (3), important for angiogenesis; and many other proteins involved in other protumorigenic processes (3–5).

Fat intake (specifically, intake of saturated or n-6 polyunsaturated fatty acids) has been associated with increased risk of colorectal adenoma (6–9). Conversely, high consumption of fish (containing n-3 fatty acids) may be protective (7,9–15). In one study, dietary supplementation with eicosapentaenoic acid (EPA; an n-3 fatty acid found in fish) in patients with colorectal adenoma led to reduced cell proliferation and increased apoptosis in the colonic mucosa (11). Because antiapoptotic proteins are downstream targets of the WNT/β-catenin pathway (3), these data support the hypothesis that dietary fat intake, specifically n-3 fatty acids found in fish or n-6 fatty acids, may act on the WNT pathway to affect risk of colorectal adenoma (6–16).

Of the 19 members of the WNT protein family (17), this study focused on WNT6 and WNT10A based on existing literature, suggesting a link between these two proteins and colorectal neoplasia. It is believed that the epithelium formation, adhesion, and cell–cell communication functions associated with WNT6 are mediated through the canonical (WNT/β-catenin signaling) pathway (5,18). Kirikoshi et al. reported that WNT6 and WNT10A are strongly co-expressed in the SW480 colorectal cancer cell line (19) and further reported that WNT10A expression was upregulated in primary gastric cancer, but WNT6 expression was not (20).

To date, we are not aware of any studies of polymorphisms in WNT6 or WNT10A in relation to colorectal neoplasia risk. We selected tagSNPs to capture common genetic variations in WNT6 and WNT10A in Caucasians; these tagSNPs were analyzed in a colonoscopy-based case-control study of colorectal polyps. We also investigated potential effect modification by fish, fat, and saturated fat intake.

MATERIALS AND METHODS

Study Population

Participant recruitment has been previously described (21–23). Briefly, adenoma cases and polyp-free controls were recruited through a large multiclinic gastroenterological practice in the Twin Cities area of Minnesota from April 1991 to April 1994. Eligibility criteria have been described elsewhere (21); participants were aged 30–74 yr, English-speaking residents of the Twin Cities metropolitan area with no known genetic syndrome associated with increased risk of colon neoplasia and no individual history of cancer (except nonmelanoma skin cancer), prior colorectal polyps, or inflammatory bowel disease. Information on diet, physical activity, anthropometrics, demographics, and medical history was obtained via questionnaire. The participation rate for all colonoscoped patients was 68%.

Questionnaire Data

Questionnaires included information on dietary intake, physical activity, smoking habits, anthropometric measurements, medical history, demographic information, reproductive history (women), and family history of polyps and cancer. The dietary questionnaire was adapted from the Willett Semi-Quantitative Food Frequency Questionnaire, which has been evaluated for validity and repeatability within this study (24,25), as well as the Nurses' Health Study cohort (26), the Iowa Women's Health Study cohort (27), and the Health Professionals Follow-Up Study cohort (28).

TagSNP Selection and Genotyping

TagSNPs for this study were selected using the Genome Variation Server (GVS) (29) with a cutoff minor allele frequency of 4% and an r^2 value of 0.90 from HapMap data release #16c. 1, June 2005, on NCBI B34 assembly, dbSNP b124. This yielded 2 SNPs each in WNT6 (rs6747776, rs6754599) and WNT10A (rs7349332, rs10177996). SNPs were genotyped using Illumina GoldenGate bead-based genotyping technology at the Translational Genomics Research Institute (TGen, Phoenix, AZ). Intra-and interplate replicates at a rate of 5% were included on all plates and in all batches. Blinded duplicates were also included on all plates as another quality control measure. DNA from CEPH trios (Coriell Cell Repository, Camden, NJ) that were genotyped by the HapMap project were included on all plates to confirm the reliability and reproducibility of the genotyping. Genotypes were excluded from analyses by TGen if any of the following were true: GenTrain Score <0.4, 10% GC Score <0.25, AB T Dev >0.1239, Call Frequency <0.85, Replicate Errors >2, P-P-C Errors >2. Further exclusions were made for SNPs that had <85% concordance with blinded or nonblinded duplicates. The 4 SNPs included in the present analyses passed all quality control measures and were in Hardy-Weinberg equilibrium among controls (all $P > 0.05$).

Statistical Analyses

Unconditional logistic regression analysis was used to examine the association of polymorphisms in WNT6 and WNT10A with colorectal adenoma incidence compared to polyp-free controls. Differences in allele frequencies between Caucasians and African

Americans were observed; therefore, analyses were restricted to Caucasians (97.2% of the study population). We considered both dominant (heterozygous and homozygous minor allele genotypes grouped together) and unrestricted (indicator variables for the heterozygous group and the homozygous minor allele group) models. Multivariate models for main associations were adjusted for age and sex. To obtain *P* trend, SNPs were included in logistic models as a continuous variable. All statistical analyses were conducted using SAS v. 9 (SAS Institute, Inc., Cary, NC). A *P* value ≤0.05 was interpreted as statistically significant; a *P* value >0.05 and ≤0.10 as marginally significant.

Effect modification with dietary intakes of fat and fish were evaluated by the inclusion of multiplicative interaction terms in logistic regression models. Tests of interaction were conducted by 1) a likelihood ratio test comparing a model that contained interaction terms to a model that did not contain interaction terms, and 2) evaluating differences in slopes within strata of the dietary factor across genotypes (e.g., comparing the slope of association across genotypes between individuals with low fat intake with the slope of those with high fat intake). Dietary variables were included in models as 1) approximate tertiles and 2) dichotomized at the median value, based on the distribution in the control population. Interaction models were adjusted for age, sex, hormone replacement therapy use, pack-years of smoking, body mass index, average daily caloric intake, and NSAID use.

RESULTS

Main Associations

Relevant characteristics of the study population are presented in Table 1. Overall, cases were older, more likely to be male, more likely to smoke tobacco, drink alcohol, and consume more calories, fat, and saturated fat than the controls. The proportion of calories from fat was greater among cases than controls. The controls were more likely to have regularly used NSAIDs and to have been on hormone replacement therapy.

Results of all main association analyses are presented in Table 2. The two SNPs in WNT6 were in low LD (r^2 =0.43) among the Caucasian controls, although the two SNPs in WNT10A were in higher LD (r^2 = 0.73). The WNT6 rs6747776 variant CC genotype was associated with increased colorectal adenoma risk compared to the GG genotype (OR: 2.56, 95% CI: 1.01–6.51; *P* trend = 0.06). No other statistically significant main associations were observed.

Interaction Analyses

WNT6

Proportion of Calories From Total Fat: The association of the WNT6 rs6747776 polymorphism with risk of adenoma was modified by an individual's proportion of calories from fat (*P* interaction = 0.03; Table 3); the greatest increase in risk was observed among those with one or more minor alleles in the lowest strata of fat intake (OR: 2.76, 95% CI: 1.49–5.09). The same pattern was observed when fat intake was dichotomized (*P* interaction = 0.02).

Fish Intake (times/wk): A marginally statistically significant interaction was observed between dichotomous weekly fish intake (a source of n-3 fatty acids) and rs6747776 (*P* interaction = 0.09; Table 3). The greatest risk was observed among individuals with at least 1 minor allele in the lower stratum of weekly fish consumption (OR: 1.66, 95% CI: 1.02–2.69), whereas no statistically significant increase in risk was seen in the stratum of high fish consumption.

No statistically significant interactions were observed between either polymorphism in WNT6 and proportion of calories from saturated fat.

WNT10A

Proportion of Calories From Saturated Fat: We observed a marginally significant interaction between WNT10 rs10177996 (intron 1) and an individual's proportion of calories from saturated fat (*P* interaction = 0.07, Table 4).

In the lowest tertile of saturated fat intake, having at least 1 minor allele was associated with a suggested increase in adenoma risk (OR: 1.70, 95% CI: 0.97–2.97). However, in the upper tertile of fat intake, both genotype groups were associated with an approximate 1.5-fold increase in adenoma risk compared to the group in the lowest tertile of fat intake with the homozygous major genotype.

DISCUSSION

To our knowledge, this is the first study evaluating WNT polymorphisms in relation to risk of colorectal adenoma. We observed that the WNT6 rs6747776 (intron 1) homozygous minor allele genotype was associated with increased risk of colorectal adenoma. Further, we detected a statistically significant interaction between this polymorphism and proportion of calories from fat. Finally, there was a suggested interaction between *WNT10A* rs10177996 (intron 1) and percent of calories from saturated fat.

WNT signaling has previously been found to mediate the procarcinogenic effects of fat intake. Fujise et al. reported that high intakes of corn oil and beef tallow were associated with increased cell proliferation mediated through the WNT pathway (6); an increase in mucosal apoptosis was associated with diets high in olive or fish oil (6,30). These changes in levels of apoptosis were associated with changes in the expression patterns of the WNT proteins in the mouse colon tissue (6). Our hypothesis was that the greatest adenoma risk would be observed for those with minor WNT alleles who consumed a high level of dietary fat. However, in this study, across all reported interactions, the greatest risk was observed among those with at least 1 minor allele and the lowest fat intake. These results are counter to our hypothesis and contrary to previous findings in colon cancer (31). Generally, we saw an increase in risk of adenoma with increasing fat intake, but the opposite association with genotypes containing at least 1 minor allele suggests that the effect of fat may vary by genotype.

Although these findings suggest an association between WNT6 rs6747776 and risk of adenoma, it remains unclear whether this polymorphism is itself functional or, rather, is in

linkage disequilibrium (LD) with a functional variant. WNT6 is involved in many important developmental processes, including embryogenesis, epithelial formation, and cell–cell communication (1,5,18,32), so it is unlikely that a deleterious mutation would persist within a population without a compensating advantage. Thus, it is not surprising that no nonsynonymous SNPs have been identified in Caucasians (29). Because rs6747776 is intronic, it is unlikely that this polymorphism affects protein structure, but it could be involved in binding transcription factors due to its location in intron 1. A more probable explanation, however, for the strong association with colorectal adenoma risk is that the rs6747776 SNP is in LD with a functional polymorphism. We used the Genome Variation Server (29) to view all SNPs 100 KB up and downstream from WNT6 to examine whether any known or likely functional SNP is in LD with rs6747776. We identified an intergenic polymorphism (rs691574 C > T) 17,852 base pairs upstream of WNT6. Using the UCSC Genome Browser (http://genome.ucsc.edu/), we identified its location in a potential enhancer site for WNT6 and possibly WNT10A (33); this region is highly conserved across species (conservation score = 0.998). However, the LD between this and the tagSNP studied here is moderate ($r^2 = 0.31$).

A potential limitation of this study was that SNP selection took place before the HapMap 3 data were available. To compare our coverage to the currently available SNP data, we used the Genome Variation Server to determine what would be selected if we were to pick tagSNPs for WNT6 and WN10A within the current HapMap build (Caucasians) (29). With current information, we would need to genotype 1 additional SNP in WNT10A and 2 SNPs in WNT6 to ensure full coverage according to our selection criteria. However, only in the case of WNT6 does any of the additional SNPs tag for any SNP other than itself. For that bin, there are only 2 SNPs genotyped by HapMap, and both are intronic, indicating that they are unlikely to be significant sources of functional genetic variability in WNT6.

Another potential limitation is our power to detect interactions between these tagSNPs and dietary factors such as fat and fish intake. However, even with this limitation, we detected several interactions between WNT6 rs6747776 and various forms of fat intake. It will be important to replicate these findings and also evaluate interactions separating out the homozygous minor allele WNT6 genotype from individuals with heterozygote genotypes.

Strengths of this study include the requirement of a full colonoscopy prior to entry among both cases and the control group. Because adenomas are common among older adults in the United States (approximately 1 in 3 adults over the age of 60) (34), and only around 30% of adults over 50 have endoscopic examinations (35), the likelihood of undiagnosed polyps in an unscreened population is high. By ensuring that all participants had a full colonoscopy, this source of misclassification was eliminated.

Overall, this study supports the involvement of genetic variability in WNT proteins in the development of colorectal polyps and provides additional evidence for an interaction between fat intake relating to colorectal adenoma risk. However, additional studies are needed to confirm our findings.

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TABLE 1

Selected characteristics of the Minnesota Cancer Prevention Research Unit Polyp Study population (restricted to Caucasians) presented as *N* (%) or mean (+/− SD)

	Polyp Study Population (*N* = 1147)		
Characteristic	**Adenomas**	**Controls**	**χ^2/*t*-test *P* Value**
N	518	629	
Age	57.9 (9.6)	52.9 (11.0)	<0.01
Age category			
<40	22 (4.3)	82 (13.0)	
40–49	80 (15.4)	163 (25.9)	
50–59	166 (32.1)	198 (31.5)	
60–69	191 (36.9)	143 (22.7)	
70+	59 (11.4)	43 (6.8)	<0.01
Sex			
Female	193 (37.3)	388 (61.7)	<0.01
Aspirin use			
Yes	147 (28.4)	193(30.7)	0.40
Any NSAID use			
Yes	187 (36.2)	271 (43.2)	0.02
Total fat intake (g/day)	74.3 (+/− 34.1)	68.7 (+/− 30.0)	<0.01
Percent of calories from fat	31.4 (+/− 6.6)	30.5 (+/− 6.4)	0.02
Saturated fat intake (g/day)	25.7 (+/− 12.8)	23.9 (+/− 11.5)	0.01
Percent of calories from saturated fat	10.8 (+/− 3.0)	10.5 (+/− 2.7)	0.10
Calorie intake (kcal/day)	2106 (+/− 771)	2011 (+/− 709)	0.03
Fish intake (times/wk)	1.9 (+/− 1.5)	1.8 (+/− 1.6)	0.78
Fiber intake (g/day)	21.8 (+/− 9.5)	21.7 (+/− 9.7)	0.81
Folate intake	1.1 (+/− 1.3)	1.1 (+/− 1.5)	0.53
Alcohol consumption (g/day)	10.3 (+/− 16.8)	6.5 (+/− 13.4)	<0.01
Pack-years			
0	172 (34.1)	301 (49.0)	
1–25	158 (31.3)	186 (30.3)	
>25	175 (34.7)	127 (20.7)	<0.01
Body mass index	27.3 (+/− 4.7)	26.9 (+/− 5.0)	0.22
Ever on HRT	76 (14.9)	193 (31.3)	<0.01
WNT6 rs6747776 (Intron 1)			
C	15.6	13.6	
G	84.4	86.4	0.21
WNT6 rs6754599 (Intron 1)			
C	14.9	13.1	
G	85.1	86.9	0.52
WNT10A rs7349332 (Intron 3)			
A	12.2	13.0	

	Polyp Study Population (*N* = 1147)		
Characteristic	**Adenomas**	**Controls**	**χ^2/*t*-test *P* Value**
G	87.8	87.0	0.24
WNT10A rs10177996 (Intron 1)			
A	83.1	83.1	
G	16.9	16.9	0.83

TABLE 2

Results of the main association analyses for colorectal adenoma risk by *WNT6* and *WNT10A* genotype

SNP	Genotype	Cases	Controls	OR (95% CI)	*P* Trend
		WNT6			
rs6747776	GG	347	429	1.00 (ref)	
G>C	GC	123	142	1.17 (0.87–1.57)	
Intron 1	CC	14	8	2.56 (1.01–6.51)	0.06
	GG	347	429	1.00 (ref)	
	GC or CC	137	150	1.24 (0.93–1.65)	NA
rs6754599	GG	351	437	1.00 (ref)	
G>C	GC	122	132	1.19 (0.88–1.61)	
Intron 1	CC	11	10	1.58 (0.63–3.95)	0.15
	GG	351	437	1.00 (ref)	
	GC or CC	133	142	1.22 (0.91–1.63)	NA
		WNT10A			
rs10177996	AA	336	399	1.00 (ref)	
A>G	AG	132	164	0.99 (0.74–1.31)	
Intron 1	GG	16	16	1.42 (0.67–2.97)	0.66
	AA	336	399	1.00 (ref)	
	AG or GG	148	180	1.02 (0.78–1.35)	NA
rs7349332	GG	371	441	1.00 (ref)	
G>A	GA	103	126	1.00 (0.73–1.37)	
Intron 3	AA	7	12	0.73 (0.27–1.96)	0.76
	GG	371	441	1.00 (ref)	
	GA or AA	110	138	0.98 (0.72–1.32)	NA

TABLE 3

Interaction between the rs6747776 G>C polymorphism in *WNT6* with percent calories from fat, and weekly fish intake

WNT6	**Proportion of Calories From Fat***			
rs6747776	**Tertile 1**	**Tertile 2**	**Tertile 3**	***P*-int¥**
GG	1.00 (ref)	1.72 (1.15–2.59)	1.63 (1.08–2.46)	
	75/154	134/138	138/137	
GC or CC	2.76 (1.49–5.09)	1.53 (0.88–2.64)	1.50 (0.89–5.50)	**0.03**
	42/37	41/52	54/61	
	Proportion of Calories From Fat**			
	Low		High	*P*-int¥
GG	1.00 (ref)		1.43 (1.03–1.98)	
	132/228		215/201	
GC or CC	1.82 (1.13–2.92)		1.27 (0.83–1.93)	**0.02**
	63/62		74/88	
	Fish Intake (times/wk)***			
	Low		High	*P*-int¥
GG	1.00 (ref)		1.29 (0.93–1.80)	
	139/196		208/233	
GC or CC	1.66 (1.02–2.69)		1.25 (0.82–1.92)	**0.09**
	64/54		73/96	

* Tertile 1: < = 27, Tertile 2: >27 and <33, Tertile 3: > = 33 (% of total calories/day).

** Low: <30, High: > = 30 (% of total calories/day).

*** Low: <1.5, High: > = 1.5 (times/wk).

¥ Adjusted for age, sex, race, BMI, hormone use, pack-years smoking, daily caloric intake, and NSAID use.

P-int = *P* interaction. Bold indicates marginally or statistically significant interaction.

TABLE 4

Interaction between genotypes the rs7349332 polymorphism in *WNT10A* and percent of calories from saturated fat

WNT10A	**Proportion of Calories From Saturated Fat***			
rs10177996	**Tertile 1**	**Tertile 2**	**Tertile 3**	***P*-int¥**
AA	1.00 (ref)	1.27 (0.84–1.91)	1.47 (0.96–2.26)	
	91/138	123/143	122/118	
AG or GG	1.70 (0.97–2.97)	0.90 (0.54–1.50)	1.47 (0.88–2.47)	**0.07**
	42/46	46/74	60/60	

*Tertile 1:< = 9, Tertile 2:>9 and <11.5, Tertile 3:> = 11.5 (% of total calories/day).

¥Adjusted for age, sex, race, BMI, hormone use, pack-years smoking, daily caloric intake, and NSAID use.

P-int = *P* interaction. Bold indicates marginally or statistically significant interaction.