

# Associations among *IRS1*, *IRS2*, *IGF1*, and *IGFBP3* Genetic Polymorphisms and Colorectal Cancer

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## Abstract

**Introduction:** Insulin, insulin-like growth factor (IGF), and IGF binding protein (IGFBP) are involved in cell growth and proliferation and are thought to be important in the etiology of colorectal cancer. We hypothesize that genetic polymorphisms of insulin receptor substrates (*IRS-1* and *IRS-2*), IGF-I, and IGFBP-3 alter colorectal cancer risk because of their roles in the insulin-related signaling pathway. **Methods:** Data from a population-based incident case-control study of 1,346 colon cancer cases and 1,544 population-based controls and 952 rectal cancer cases and 1,205 controls were used to evaluate associations. Genetic polymorphisms of four genes were investigated: an *IGF1 CA* repeat, the *IGFBP3* -202 A > C, the *IRS1 G972R*, and the *IRS2 G1057D*. **Results:** Having at least one *R* allele (*GR* or *RR*) for *IRS1 G972R* was associated with an increased risk of colon cancer [odds ratio 1.4, 95% confidence interval (95% CI) 1.1-1.9]. The *IRS2 G972R* heterozygote *GD* genotype significantly reduced risk of colon cancer (odds ratio 0.8, 95% CI 0.6-

0.9). Neither the *IGF1* nor the *IGFBP3* variants was associated independently with colon cancer, but there was an association when examined with *IRS1*. Individuals with an *IRS1 R* allele and *IGF1 non-192* allele were at a 2-fold increased risk of colon cancer (95% CI 1.2-4.4). There was a 70% (95% CI 1.02-2.8) increased risk of colon cancer with an *IRS1 R* allele and the *IGFBP3 AC* or *CC* genotype. The *IRS2 GD* genotype reduced risk of colon cancer, except among those with an *IRS1 R* allele. No significant associations were seen in analyses of main effects or interactions of these variants and rectal cancer risk. **Conclusions:** Both *IRS1* and *IRS2* variants were associated with colon cancer risk independently. Associations were slightly stronger when polymorphisms in multiple genes were evaluated in conjunction with other genes rather than individually. These data suggest that the insulin-related pathway may be important in the etiology of colon cancer but not rectal cancer. (Cancer Epidemiol Biomarkers Prev 2004; 13(7):1206-14)

## Introduction

Uncontrolled cell growth is central to the carcinogenic process. Factors that regulate and control cell growth are undoubtedly important to the etiology of cancer. While the biological rationale for the importance of looking at factors influencing cell growth is clear, determination of which factors may be most informative is not. There is a growing body of evidence that suggests that insulin-like growth factors (IGF), IGF binding proteins (IGFBPs), especially IGFBP-3, and insulin play a significant role in the initiation of cell growth and proliferation of colorectal cancers (1-5). Insulin has been regarded as primarily a metabolic signal, while IGF-I has been implicated as an important mitogen and cell differentiation factor (6, 7). The insulin receptor substrate (*IRS*) protein family contains several members, of which *IRS-1* and *IRS-2* are

expressed in almost all cells and tissues (8-10). While *IRS-1* controls body growth and peripheral insulin action, *IRS-2* regulates body weight control and glucose homeostasis (9). Within tumors, *IRS-1* may be a marker of an active IGF signal transduction pathway (4, 11), although *IRS* is involved in insulin signaling.

Many factors are involved in the regulation of insulin and IGFs, including diet, lifestyle, hormonal, and genetic factors. Studies have shown that diet, physical activity, body size, and sex steroids are involved in the regulation of these hormones (12-18); some data suggest that serum levels of IGF, IGFBP-3, and *IRS* also may be affected by polymorphisms in these genes (19-22). Now, it is somewhat uncertain which variants in these genes are most relevant in the regulation of serum levels of IGF-I, IGFBP-3, *IRS-1*, and *IRS-2* and in the etiology of colorectal cancer. Few variants of these genes have been evaluated with cancer, most notably a *CA* repeat of *IGF1* (23-26), with inconsistent results. We could find no studies assessing associations with any variants of these genes and colorectal cancer. However, based on the literature, there are indications that certain genetic polymorphisms may be important.

The *G972R* polymorphism in *IRS1* has been associated with insulin resistance and type 2 diabetes, leading to the hypothesis that the *IRS1 R* allele would increase risk of

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colon cancer (27). The *IRS2* G1057D polymorphism has been associated with insulin resistance, although the association has been shown to differ based on body size (28). High IGF-I and low IGFBP-3 serum levels have been associated with colon cancer risk in some but not all studies. Variation in serum IGF-I levels has been associated with a polymorphism in the *IGF1* gene 1 kb upstream of the transcription start site (29). This is a CA repeat polymorphism, with the most common allele containing 19 CA repeats, denoted "192" for the size of the PCR product (29). Because IGF-I serum levels have been shown to be lower among men with the 192/192 genotype, one could hypothesize that the 192/192 genotype would decrease colorectal cancer risk. In *IGFBP3*, an A > C polymorphism at nucleotide -202 has been associated with different levels of IGFBP-3 in a dose response fashion (i.e., AA > AC > CC; ref. 30). Therefore, one could predict that the AC or CC genotypes could be associated with an increased risk of colorectal cancer, as some studies indicate that high levels of IGFBP-3 reduce colon cancer risk and those with the AC and CC genotypes should have the lowest IGFBP-3 levels.

In this article, we evaluate the associations of genetic polymorphisms in the *IGF1*, *IGFBP3*, *IRS1*, and *IRS2* genes with colorectal cancer both independently and in conjunction with each other. We hypothesize that the 192/192 *IGF1* genotype reduces risk of colorectal cancer and that the AA genotype of the *IGFBP3* gene reduces risk of colorectal cancer; the joint effects of these genotypes may be more important than each genotype independently, given that IGFBP-3 regulates bioavailability of IGF-I. We hypothesize that having a R allele of the *IRS1* gene will increase risk of colon cancer and that the DD genotype of the *IRS2* gene will decrease risk of colon cancer. We also evaluate if these genes relate to colorectal cancer risk differently among those with and without a family history of colorectal cancer. We look at family history of cancer, because there is limited information on the association of relatively common, low penetrance genes and colon cancer risk among those with and without a family history. We hypothesize that the importance of these genes may vary by family history of colorectal cancer.

## Methods

**Study Population.** Participants in the study were from the Kaiser Permanente Medical Care Program (northern California) and Utah. All eligible cases within these defined geographic areas were identified and recruited for the study. Two study populations are included in these analyses. The first study includes cases and controls from a population-based case-control study of first primary colon cancer (*International Classification of Diseases for Oncology, Second Edition* codes 18.0 and 18.2 to 18.9) diagnosed between October 1, 1991 and September 30, 1994. The second study includes cases with a first primary tumor in the rectosigmoid junction or rectum, identified between May 1997 and May 2001 in Utah and in Kaiser Permanente Medical Care Program. Case eligibility was determined by the Surveillance, Epidemiology, and End Results cancer registries

in northern California and in Utah. In both studies, cases were identified using rapid reporting systems, and eligibility included being between ages 30 and 79 years at time of diagnosis, English speaking, mentally competent to complete the interview, no previous history of colorectal cancer (31), and no known (as indicated on the pathology report) familial adenomatous polyposis, ulcerative colitis, or Crohn's disease.

Controls were matched to cases by sex and by 5-year age groups. At the Kaiser Permanente Medical Care Program, controls were randomly selected from membership lists; in Utah, controls ages  $\geq 65$  years were randomly selected from Health Care Financing Administration lists and controls ages <65 years were randomly selected from driver's license lists. The analysis includes 1,346 colon cancer cases and 1,544 controls interviewed between February 1991 and May 1994 and 952 rectal cancer cases and 1,205 controls interviewed between October 1997 and January 2002. For the colon study, 80.8% of cases and 71.6% of controls whom we were able to contact were interviewed; for the rectal study, we interviewed 73.2% of cases and 68.8% of controls contacted. Response rates, or the number interviewed over all persons identified, were 71.8% for colon cancer cases and 68.0% for controls selected for the colon cancer study and 65.2% for cases and 65.3% for controls for the rectal cancer study. The primary reasons for nonparticipation of cases were death prior to interview or too ill to be interviewed (~11%) and refusal for both cases (20%) and controls (29%).

**Questionnaire Data.** Family history of colorectal cancer was determined from an interviewer-administered questionnaire. Participants were asked to list all first-degree family members, including parents, siblings, and children. After enumerating family members, participants were asked to report family members that had ever been diagnosed with cancer and specific type of cancer they were diagnosed with. In this analysis, family history of colorectal cancer included cancer of the colon, rectum, or large bowel. Other questionnaire data included age, diet history, physical activity history, medical history, and reproductive history.

**Genotyping.** DNA was extracted from peripheral blood leukocytes. Of the colon cancer cases and controls who were interviewed, 1,181 cases and 1,194 controls had germ line DNA available for analysis; many samples were no longer available given previous analyses. Of the 952 rectal cancer cases and 1,205 controls interviewed, 827 cases and 1,031 controls had DNA extracted. Of these, genotyping data were available for 792 rectal cancer cases and 985 controls for *IGF1*, 794 rectal cancer cases and 989 controls for *IGFBP3*, 796 rectal cancer cases and 988 controls for *IRS1*, and 766 rectal cancer cases and 983 controls for *IRS2*. For quality control, known controls representing all polymorphisms and blanks were included in each 96-well tray. All genotypes were scored by two individuals to help insure accuracy.

***IRS1.*** The G972R polymorphism was detected using PCR amplification with primers 5'-CTTCTGTCAGG-TGTCCATCC-3' and 5'-TGGCGAGGTGTCCACGTAGC-3' (32). PCR cycling consisted of an initial denaturation at 94°C for 2 minutes, 10 cycles at 94°C for 10 seconds,

60°C for 10 seconds, and 72°C for 10 seconds followed by 30 cycles at 94°C for 10 seconds, 55°C for 10 seconds, and 72°C for 10 seconds. *Bst*NI was used to digest the PCR products following manufacturer's instructions. Alleles were scored as either G for glycine or R for arginine (absence or presence of the restriction site, respectively).

**IRS2.** The *G1057D* polymorphism was detected using a previously described TaqMan assay (33) with minor modifications. Primer and probe sequences for the TaqMan assay were as follows: primer IRS-2 F 5'-GGA-GCTGTACCGCCTGCC-3', primer IRS-2 R 5'-ACCAA-AAGCCATCTCGGTGT-3', G-probe FAM-CCGGGCGC-CGCCTCAT-Tamra, and A-probe VIC-CGGACGCCGC-CTCATCGTT-Tamra. Each 17  $\mu$ L PCR reaction contained 20 ng genomic DNA, 900 nmol/L of each primer, 130 nmol/L of each TaqMan probe, and 8.5  $\mu$ L TaqMan 2 $\times$  Universal PCR Master Mix (contains AmpErase UNG and AmpliTaq Gold enzymes, deoxynucleotide triphosphates, and reaction buffer). PCR was carried out under the following conditions: 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 62°C for 1 minute using IQ detection system (Bio-Rad, Hercules, CA). The fluorescence of each sample was collected and analyzed by iCycler IQ real-time detection software (version 3.0).

**IGF1.** The *IGF1 CA* repeat was amplified using PCR primers F 5'-GCTAGCCAGCTGGTATT-3' and R 5'-ACCACTCTGGGAGAAGGGTA-3' (29, 34). PCR conditions consisted of a 2-minute denaturation at 94°C followed by 30 cycles of 94°C for 10 seconds, 57°C for 10 seconds, and 72°C for 15 seconds. The *IGF1* products were electrophoresed on 6% denaturing polyacrylamide gels at 70 W for 3 hours. The gels were dried and exposed to X-ray film. Alleles were assigned by size of fragment (bp) and classified as "192" or "non-192." "192" is the PCR product size of the most common allele, which contains 19 *CA* repeats.

**IGFBP3.** The -202 A > C polymorphism was amplified using primers F 5'-CCACGAGGTACACACGAATG-3' and R3 5'-TGAGCAGCCGGGGCCGAG-3' (35). AmpliTaq Gold (0.5 units) and 5% DMSO were used to increase efficiency of amplification. PCR conditions were 9-minute initial denaturation at 95°C followed by 40 cycles at 95°C for 10 seconds and 66°C for 20 seconds. The resulting PCR product was digested with *Alw*211 (4 units) at 37°C overnight. Digested products were separated on a 2% NuSieve gel stained with ethidium bromide and visualized with UV light. Alleles were scored as either A or C (presence or absence of the restriction site, respectively).

**Statistical Methods.** *IRS1* genotypes were *GG*, *GR*, and *RR*, with the *R* allele being less common. *IRS2* genotypes were *GG*, *GD*, and *DD*, with the *GG* genotype being most common. *IGF1* genotypes were 192/192, heterozygous, or non-192 alleles; the absence of the 192 allele was less common than the presence of the 192 allele. *IGFBP3* genotypes were *CC*, *CA*, and *AA*. Tumor site was defined as proximal (cecum through transverse colon), distal (splenic flexure, descending, and sigmoid colon), and rectal (rectosigmoid junction and rectum).

SAS Statistical Package (version 8.2) was used to conduct the analyses. Analyses included evaluating the distribution of the genotypes in the population, the independent associations of genetic polymorphisms with colorectal cancer risk, and the joint effect of genotypes on colorectal cancer risk. Logistic regression models were used to assess risk associations. Because controls were frequency matched to cases, matched logistic regression models were not used. In all logistic models, age and sex were adjusted, unless data were stratified by sex, where only age was used as an adjustment variable. Other lifestyle factors, such as diet and physical activity, did not appreciably alter associations. No differences were detected by center, so center was not adjusted in analyses. Because the majority of the population was non-Hispanic White, adjustment for racial group did not influence results; stratification by ethnic group was not meaningful given small sample size for participants who were not White (see Table 1). Sex- and age-specific analyses were done; categorization of age was done for those ages  $\geq 65$  and  $< 65$  years. Several tests for interaction between genes were performed. The relative excess risk from interaction and corresponding 95% confidence interval (95% CI) was calculated to provide insight into differences that might be expected on an additive scale of relative excess risk from interaction (36). The Wald  $\chi^2$  test was used to determine significant differences in slopes. Hardy-Weinberg equilibrium and allele frequency was determined using the SAS Genetics program.

## Results

Rectal cancer cases were younger than colon cancer cases (Table 1). For both colon and rectal cancer, there were more men than women interviewed. In both studies, the population was predominately non-Hispanic White. Family history of colorectal cancer was reported in the same frequency for both colon controls and rectal controls, although more colon cancer cases than rectal cancer cases reported a history of colorectal cancer. Few differences in genotype distribution existed by study controls, where  $\sim 11\%$  had either *GR* or *RR* genotype for *IRS1*, 12% to 14% had the *DD IRS2* genotype, 40% had the 192/192 *IGF1* genotype, and 27% to 29% had the *IGFBP3 CC* genotype.

Based on data from the control population, *IGFBP3*, *IRS1*, and *IRS2* were in Hardy-Weinberg equilibrium, while *IGF1* was not (data not shown). Results were similar from controls selected for the colon study and those selected for the rectal cancer study ( $P = 0.35$  and 0.13 for *IGFBP3* for colon and rectal, respectively;  $P = 0.64$  and 0.26 for *IRS1* for colon and rectal, respectively;  $P < 0.01$  for colon and 0.07 for *IGF1*; and  $P = 0.44$  for both colon and rectal studies for *IRS2*). Allele frequencies also were similar between the two samples. The *A* allele frequency for *IGFBP3* was 0.463 (95% CI 0.448-0.479) for colon and 0.468 (95% CI 0.446-0.492) for rectal; the *C* allele frequency was 0.537 (95% CI 0.521-0.552) and 0.531 (95% CI 0.508-0.554) for colon and rectal studies, respectively. The *IRS1 G* allele frequency was 0.939 (95% CI 0.932-0.947) and 0.939 (95% CI 0.928-0.949) for colon and rectal controls, respectively; the *R* allele frequency was 0.606 (95% CI 0.531-0.680) and 0.612

**Table 1. Description of Utah and Kaiser Permanente Medical Care Program population**

	Colon		Rectal	
	Cases <i>n</i> (%)	Controls <i>n</i> (%)	Cases <i>n</i> (%)	Controls <i>n</i> (%)
Age (y)				
<50	108 (8.0)	163 (10.6)	147 (15.4)	178 (14.8)
50-64	475 (32.3)	489 (31.7)	412 (43.3)	496 (41.2)
65-79	763 (56.7)	892 (57.8)	393 (41.3)	531 (44.1)
Gender				
Men	756 (56.2)	845 (54.7)	559 (58.7)	673 (55.9)
Women	590 (43.8)	699 (45.3)	393 (41.3)	532 (44.2)
Race/ethnicity				
White	1,167 (86.8)	1,360 (88.2)	767 (81.6)	999 (83.9)
Hispanic	82 (6.1)	104 (6.7)	72 (7.7)	91 (7.6)
African American	95 (7.1)	78 (5.1)	38 (4.0)	50 (4.2)
Asian	0	0	53 (5.6)	47 (3.9)
Native American	0	0	10 (1.1)	4 (0.3)
Family history of colorectal cancer				
Yes	200 (14.9)	134 (8.7)	103 (10.8)	99 (8.2)
No	1,146 (85.1)	1,410 (91.3)	849 (89.2)	1,106 (91.8)
<i>IGF1</i>				
192/192	433 (37.0)	479 (40.9)	327 (42.1)	399 (40.5)
192/ <i>non</i> -192	567 (48.5)	533 (45.5)	348 (44.8)	450 (45.7)
<i>Non</i> -192	170 (14.5)	159 (13.6)	102 (13.1)	136 (13.8)
<i>IGFBP3</i>				
CC	303 (25.9)	340 (29.2)	221 (28.4)	267 (27.0)
AC	613 (52.4)	565 (48.6)	384 (49.3)	517 (52.3)
AA	253 (21.6)	258 (22.2)	174 (22.3)	205 (20.7)
<i>IRS1</i>				
GG	998 (84.5)	1,031 (88.4)	681 (87.2)	873 (88.4)
GR	176 (14.9)	131 (11.2)	98 (12.6)	109 (11.0)
RR	7 (0.6)	4 (0.3)	2 (0.3)	6 (0.6)
<i>IRS2</i>				
GG	467 (46.5)	481 (41.2)	325 (42.4)	421 (42.8)
GD	409 (40.7)	552 (47.3)	343 (44.8)	423 (43.0)
DD	128 (12.8)	134 (11.5)	98 (12.8)	139 (14.1)

(95% CI 0.511-0.724) for colon and rectal controls, respectively. The *IRS2* G allele frequency was 0.658 (95% CI 0.643-0.672) and 0.645 (95% CI 0.630-0.622), while the D allele frequency was 0.342 (95% CI 0.329-0.366) and 0.355 (95% CI 0.338-0.370) for colon and rectal controls, respectively. There were 13 alleles detected for *IGF1*. The 192 allele was the most common, with a frequency of 0.642 (95% CI 0.627-0.657) and 0.634 (95% CI 0.613-0.553) for the colon and rectal cancer control participants. Other common *IGF1* alleles were the 194 allele (frequency 0.184 for colon and 0.172 for rectal), the 190 allele (frequency 0.054 for colon and 0.070 for rectal), and the 196 allele (frequency 0.073 for colon and 0.080 for rectal).

Analyses were conducted separately for colon cancer and rectal cancer. For main effects, there was a significant association of the *IRS1* R allele and colon cancer (Table 2). Among women, having at least one copy of the *IRS1* R allele (i.e., GR or RR genotypes) was associated with a significantly increased risk of colon cancer. The associations for *IRS1* were significantly stronger among individuals diagnosed prior to age 65 years [data not shown; odds ratio (OR) 1.7, 95% CI 1.2-2.5; *P* interaction < 0.05]. The *IRS2* G972D heterozygous

GD genotype was associated with a significant 20% to 30% decrease in colon cancer risk overall. There were no associations of the main effects of the *IGF1* and *IGFBP3* variants and colon cancer risk. There were no significant associations between any of the variants evaluated and rectal cancer risk.

In the analyses of associations of the gene-gene interactions and colon cancer risk (Table 3), there was a significant increase in risk for individuals with an *IRS1* R allele and with at least one *IGF1 non*-192 allele (OR for 192 heterozygote 1.6, 95% CI 1.1-2.3; OR for *non*-192 alleles 2.3, 95% CI 1.2-4.4), although the *P* for interaction did not reach statistical significance (0.20). The *IRS1* GR/RR genotypes also resulted in a 70% increased risk of colon among those who had either AC or AA genotype in *IGFBP3*. Similar associations were not observed with rectal cancer (data not shown).

Assessment of associations among those with and without a family history of colorectal cancer showed that the GR/RR *IRS1* genotypes were associated significantly with an increased risk of colon cancer among those without a family history of colorectal cancer (Table 4). Having *non*-192 *IGF1* allele was associated with an increased risk of colon cancer among those with a family

history of colorectal cancer, although given the small sample size, this association was not statistically significant (OR 1.8, 95% CI 0.8-3.9). These genotypes did not appear to be importantly related to rectal cancer in people with or without a family history of colorectal cancer (data not shown).

## Discussion

It has been suggested that insulin and IGF may importantly contribute to risk of colorectal cancer. Polymorphisms in genes that involved in the regulation of serum levels of IGF, insulin, and IRS may be associated with colorectal cancer. While many genes are involved in the regulation of insulin-related factors, we assessed four polymorphisms that had been shown to have functional significance in the regulation of hormone levels and may therefore influence colorectal cancer risk. Evaluation of genetic polymorphisms in four genes along this pathway suggests that having at least one *R* allele of the *IRS1* gene (*GR/RR* genotypes) may increase risk of colon cancer. The *GR/RR* genotypes of the *IRS1* gene in combination with specific genotypes of *IGFBP3* (*AC* or *AA*) and *IGF1* (*non-192* allele) also appear to increase risk of colon cancer, while a significant inverse association was observed for those with the *IRS2 GD* genotype.

*IRS-1* is the major cytoplasmic substrate of the insulin receptor in most insulin-sensitive tissues, and some studies suggest that *IRS-1* plays an important role in regulating insulin secretion in pancreatic  $\beta$  cells (37-39).

Of the many polymorphisms of the *IRS1* gene described, the glycine-to-arginine substitution at codon 972 (*G972R*) has been studied in conjunction with obesity, polycystic ovary syndrome, and non-insulin-dependent diabetes (32, 40, 41), making this a plausible variant that may alter cancer risk. The *R* allele has been associated with impaired insulin-associated signaling (32, 41) and lower fasting plasma concentrations of insulin and C-peptides (32). Insulin resistance has been hypothesized as being associated with colon cancer (17), so a slight increase in risk associated with the *R* allele may be the result of its association with obesity, poorer insulin sensitivity, diabetes, and altered insulin action and secretion (27, 32, 42, 43). We observed that the *R* allele slightly increased risk (OR 1.4) of colon cancer overall, with a slightly stronger association for those diagnosed prior to age 65 years (OR 1.8).

*IRS2*, like *IRS1*, is thought to be involved in insulin signaling and glucose intolerance (8, 10, 44). There is little information about the *G1057D* polymorphism of the *IRS2* gene, although there is reason to believe that *IRS2* may be an important risk factor for colon cancer, given its previously reported association with obesity and diabetes (9, 28, 45). *IRS2*<sup>-/-</sup> mice have been shown to be obese (9). The *G1057D IRS2* polymorphism is a reasonable candidate to evaluate in conjunction with colon cancer given its relationship to *IRS2* haplotypes associated with obesity (28). Lautier et al. (28) also found that the *G1057D IRS2* polymorphism was heterogeneous in its association with obesity and that having the *R* allele of the *IRS1* gene influenced the association seen with the *G1057D IRS2*

**Table 2. Age-adjusted associations between genotypes and colon and rectal cancer**

	Colon			Rectal		
	Everyone	Men	Women	Everyone	Men	Women
<i>IGF1</i>						
192/192 ( <i>n</i> )*	433/479	234/273	199/206	327/399	194/228	133/171
192/ <i>non-192</i>	577/533	331/297	236/236	348/450	201/260	147/190
<i>Non-192</i>	170/159	88/76	82/83	102/136	62/68	40/68
192/192 OR (95% CI)	1.0	1.0	1.0	1.0	1.0	1.0
192/ <i>non-192</i>	1.2 (1.0-1.4)	1.3 (1.0-1.6)	1.0 (0.8-1.4)	1.0 (0.8-1.2)	0.9 (0.7-1.2)	1.0 (0.7-1.4)
<i>Non-192</i>	1.2 (0.9-1.5)	1.4 (0.9-1.9)	1.0 (0.7-1.5)	0.9 (0.7-1.2)	1.1 (0.7-1.6)	0.7 (0.5-1.2)
<i>IGFBP3</i>						
CC ( <i>n</i> )	303/340	153/176	150/164	221/267	120/146	101/121
AC	613/565	355/320	258/245	384/517	238/299	146/218
AA	253/258	143/143	110/115	144/205	99/114	75/91
CC OR (95% CI)	1.0	1.0	1.0	1.0	1.0	1.0
AC	1.2 (1.0-1.5)	1.3 (1.0-1.7)	1.2 (0.9-1.5)	0.9 (0.7-1.1)	1.0 (0.7-1.3)	0.8 (0.6-1.1)
AA	1.1 (0.9-1.4)	1.1 (0.8-1.6)	1.0 (0.7-1.5)	1.0 (0.8-1.4)	1.1 (0.7-1.5)	1.0 (0.7-1.5)
<i>IRS1</i>						
GG ( <i>n</i> )	998/1,031	557/562	441/469	681/473	397/496	284/377
GR/RR	183/135	99/80	84/55	100/115	62/64	38/51
GG OR (95% CI)	1.0	1.0	1.0	1.0	1.0	1.0
GR/RR	1.4 (1.1-1.9)	1.3 (0.9-1.7)	1.6 (1.1-2.3)	1.1 (0.8-1.5)	1.2 (0.8-1.7)	1.0 (0.6-1.5)
<i>IRS2</i>						
GG ( <i>n</i> )	467/481	264/267	203/214	325/421	194/232	131/189
GD	409/552	234/303	175/249	343/423	193/247	150/176
DD	128/134	79/70	49/64	98/139	67/79	31/60
GG OR (95% CI)	1.0	1.0	1.0	1.0	1.0	1.0
GD	0.8 (0.6-0.9)	0.8 (0.6-1.0)	0.7 (0.6-1.0)	1.0 (0.9-1.3)	0.9 (0.7-1.2)	1.2 (0.9-1.7)
DD	1.0 (0.7-1.3)	1.1 (0.8-1.6)	0.8 (0.5-1.2)	0.9 (0.7-1.2)	1.0 (0.7-1.5)	0.7 (0.4-1.2)

\**n*, cases/controls.

**Table 3. Joint effects of genetic polymorphisms on colon cancer risk**

	<i>IGF1</i>			<i>P</i> (Relative Excess Risk from Interaction, Wald $\chi^2$ )
	<i>192/192</i>	<i>192/Non-192</i>	<i>Non-192</i>	
<i>IGFBP3</i>				
CC ( <i>n</i> )	122/130	132/171	43/37	0.64, 0.02
AC	216/230	293/254	90/78	
AA	88/113	122/102	34/41	
CC OR 95% CI	1.0	0.8 (0.6-1.2)	1.2 (0.7-2.1)	
AC	1.0 (0.7-1.4)	1.2 (0.9-1.7)	1.2 (0.8-1.8)	
AA	0.8 (0.6-1.2)	1.3 (0.9-1.8)	0.9 (0.5-1.5)	
<i>IRS1</i>				
GG ( <i>n</i> )	372/422	462/461	138/143	0.20, 0.34
GR/RR	56/55	93/65	28/14	
GG OR (95% CI)	1.0	1.1 (0.9-1.4)	1.1 (0.8-1.4)	
GR/RR	1.2 (0.8-1.7)	1.6 (1.1-2.3)	2.3 (1.2-4.4)	
<i>IRS2</i>				
GG ( <i>n</i> )	171/190	223/217	70/70	0.74, 0.90
GD	163/228	190/256	51/64	
DD	44/58	62/55	21/21	
GG OR (95% CI)	1.0	1.1 (0.9-1.5)	1.1 (0.8-1.7)	
GD	0.8 (0.6-1.1)	0.8 (0.6-1.1)	0.9 (0.6-1.4)	
DD	0.8 (0.5-1.3)	1.2 (0.8-1.9)	1.1 (0.6-2.1)	
<i>IGFBP3</i>				
	CC	AC	AA	
<i>IRS1</i>				
GG ( <i>n</i> )	250/296	507/497	211/230	0.64, 0.92
GR/RR	48/43	96/66	35/25	
GG OR (95% CI)	1.0	1.2 (1.0-1.5)	1.1 (0.8-1.4)	
GR/RR	1.3 (0.8-2.1)	1.7 (1.2-2.5)	1.7 (1.02-2.8)	
<i>IRS2</i>				
GG ( <i>n</i> )	131/140	236/229	99/107	0.85, 0.78
GD	111/167	208/263	84/118	
DD	31/31	61/68	35/33	
GG OR (95% CI)	1.0	1.1 (0.8-1.5)	1.0 (0.7-1.4)	
GD	0.7 (0.5-1.0)	0.8 (0.6-1.1)	0.8 (0.5-1.1)	
DD	1.1 (0.6-1.8)	1.0 (0.6-1.4)	1.1 (0.7-1.9)	
<i>IRS2</i>				
	GG	GD	DD	
<i>IRS1</i>				
GG ( <i>n</i> )	392/425	346/483	111/117	0.16, 0.22
GR/RR	71/51	61/65	14/17	
GG OR (95% CI)	1.0	0.8 (0.6-0.9)	1.0 (0.8-1.4)	
GR/RR	1.5 (1.0-2.2)	1.0 (0.7-1.5)	0.9 (0.4-1.8)	

NOTE: Adjusted for age and sex.

gene and obesity. In our study, we observed that people with the *GD* genotype were at reduced risk of colon cancer, with stronger associations among women, although neither *GD* nor *DD* genotype was associated with significant altered rectal cancer risk in either men or women.

IGF-I may be important for colorectal cancer risk because of its role in cell growth and differentiation (34, 46, 47). High IGF-I serum levels have been associated with increased risk of colorectal cancer (relative risk 2.51, 95% CI 1.15-5.46; ref. 48). In one study, high levels of IGF-I were associated with a 5-fold increase in risk of colorectal cancer (48, 49). Laboratory studies also have shown the presence of IGF-I receptors in normal intestine (suggesting a role for normal growth and function of intestinal cells) and colorectal cancer cell lines (2, 50, 51). Variation in serum IGF-I levels has been associated with a polymorphism 1 kb upstream of the transcription start site of the *IGF1* gene (29). For men, serum IGF-I concentrations were lower with the *192/192* genotype than

for other genotypes. One report showed that individuals who were *194/192* heterozygotes had 25% higher IGF-I serum levels than those who were homozygous for the *192* alleles. In our study, the *192/192* genotype did not affect the risk of colon cancer by itself, although the lack of the *192* allele did appear to increase colon cancer risk in combination with the *IRS1 R* allele. This is consistent with the data showing that the lack of the *192* allele is associated with increased IGF-I levels, which in turn is associated with an increased cancer risk.

IGFBP-3 modulates the activity of IGF-I (5, 52). In human colorectal cancer cell lines, studies have shown that binding protein levels may dictate growth response to IGF-I (2, 53, 54). High levels of IGFBP-3 have been associated with reduced risk of colorectal cancer (relative risk 0.28, 95% CI 0.12-0.66; ref. 30). IGFBP-3 also appears to play a role in regulation of cancer cell growth, independent of the IGF signaling pathway, through inducing apoptosis (52, 55). Sequence alterations have been reported in the *IGFBP3* gene in a significant

proportion of gastrointestinal tumors (22). The  $-202 C > A$  transition has been shown to result in different levels of IGFBP-3 in a dose response fashion (i.e.,  $AA > AC > CC$ ). We observed little variation in risk associated with the *IGFBP3*  $-202 C > A$  polymorphism for colon cancer independently, although there was an association in conjunction with the *IRS1 R* allele, where those with the *AC* or *CC* genotype were at greater risk if they also had an *IRS1 R* allele. Although the *IGFBP3* polymorphisms did not appear to affect cancer risk independently, the effect of the *C* allele in conjunction with the *IRS1* genotype is in the direction predicted by the effect of this allele on IGFBP-3 levels.

These data suggest that genes involved in the insulin-related pathway may be more important for colon cancer than for rectal cancer, because we saw no effect of these polymorphisms on rectal cancer risk. Significant associations of the *G972R* variant and colon cancer were observed alone and in association with the *IGFBP3* and *IGF1* variants. However, for rectal cancer, we observed little variation in risk for any of the variants whether alone or in combination. Support for a weaker role of the insulin-related pathway for rectal cancer than for colon cancer also exists in the epidemiology literature, where obesity generally is not associated with rectal cancer (56, 57) but is associated with colon cancer (31); physical activity is more consistently associated with colon than rectal cancer. Dietary glycemic index has been associated with colon cancer (58, 59), although less is known about the association between rectal cancer and dietary components that may be related to insulin.

**Table 4. Age- and sex-adjusted associations between genotypes and colon cancer by family history of colorectal cancer**

Family History	No	Yes
<i>IGF1</i>		
192/192 ( <i>n</i> )	365/436	68/43
192/ <i>non</i> -192	489/485	78/48
<i>Non</i> -192	139/148	31/11
192/192 OR (95% CI)	1.0	1.0
192/ <i>non</i> -192	1.2 (1.0-1.5)	1.0 (0.6-1.8)
<i>Non</i> -192	1.1 (0.9-1.5)	1.8 (0.8-3.9)
<i>IGFBP3</i>		
<i>CC</i> ( <i>n</i> )	251/319	52/21
<i>AC</i>	526/511	87/54
<i>AA</i>	215/234	38/24
<i>CC</i> OR (95% CI)	1.0	1.0
<i>AC</i>	1.3 (1.1-1.6)	0.7 (0.4-1.2)
<i>AA</i>	1.2 (0.9-1.5)	0.6 (0.3-1.3)
<i>IRS1</i>		
<i>GG</i> ( <i>n</i> )	854/948	144/83
<i>GR/RR</i>	154/116	29/19
<i>GG</i> OR (95% CI)	1.0	1.0
<i>GR/RR</i>	1.5 (1.1-1.9)	0.8 (0.4-1.5)
<i>IRS2</i>		
<i>GG</i> ( <i>n</i> )	396/442	71/39
<i>GD</i>	341/505	68/47
<i>DD</i>	108/119	20/15
<i>GG</i> OR (95% CI)	1.0	1.0
<i>GD</i>	0.7 (0.6-0.9)	0.8 (0.5-1.3)
<i>DD</i>	1.0 (0.8-1.4)	0.8 (0.3-1.6)

A major strength of the study is our inclusion of several variants in several genes along an insulin-related pathway that may be associated with colorectal cancer. The large size of our study allows us to study multiple variants; although given the rare frequency of some alleles, we still are limited in our ability to assess statistically significant associations. Focusing on a disease pathway rather than isolated genetic polymorphisms along that pathway allows for a more complete examination of the pathway and interpretation of results. We believe that looking at multiple genes along a pathway provides more information on the importance of the pathway to the etiology of the disease. A limitation of this study is that we examined only four polymorphisms in four genes along this pathway; one of the genes evaluated was not in Hardy-Weinberg equilibrium. This most likely suggests that many of the alleles are rare given that *IGF1* is a length variant and that there is sampling variation for rare genotypes such as the *non*-192/192 *IGF-1*. Although these polymorphisms have been reported to show associations with hormone levels and/or clinic parameters, there may be other polymorphisms in these and other genes in this pathway that importantly regulate insulin, IGF, and IRS and may be involved in the carcinogenic process. It will be important in the future to investigate other functional polymorphisms of genes along an insulin-related pathway to further our understanding of insulin in the etiology of colon cancer.

A second limitation is the lack of ethnic diversity in the population study, although ethnic distribution was similar between cases and controls. These findings may be most applicable to non-Hispanic White populations, as it is known that there is ethnic variation in allele frequency. For example, a study evaluating the *IGF1 CA* repeat showed different genotype frequencies based on ethnic background: 33.9% of 123 African Americans had *non*-192/192 alleles, 32.0% of 71 Japanese Americans had *non*-192/192 alleles, 6.4% of 58 non-Latino Whites had *non*-192/192 alleles, and 14.7% of 154 Latino Americans had *non*-192/192 *IGF1* alleles (25). In our control population, ~14% had no *IGF1 non*-192 alleles. One study examining the *IGFBP3*  $-202$  polymorphism in 943 mostly non-Hispanic White women observed the *AA* genotype in 21.6% (60); a study of 478 men in the Physician's Health Study showed that 25% had the *AA IGFBP3* genotype (20). In our study, ~21% of controls had the *AA IGFBP3* genotype. Likewise, our estimate of ~12% of controls having a *R* allele of the *IRS1* gene is lower than was observed in 157 subjects whose body mass index was  $>30$  kg/m<sup>2</sup> (~20%) but higher than observed in 157 subjects with a body mass index of  $<28$  kg/m<sup>2</sup> (8.3%; ref. 42). Information from this study adds to our knowledge of the frequency of these variants in the population, given the limited number of studies of these variants, many of which are not population based or are limited in number of individuals with available genetic data. A major strength is our ability to reconfirm allele frequencies of these genes from two large control samples that were collected at two different points in time.

In conclusion, polymorphisms in *IRS1* and *IRS2* have modest independent effects on colon cancer risk. However, when the *IRS1*, *IRS2*, *IGF1*, and *IGFBP3* variants are evaluated together, a more substantial and

significant effect on colon cancer risk is observed. These data provide some support for an insulin pathway in the etiology of colon but not rectal cancer. As with all such studies, confirmation of our results in other populations is necessary to establish their significance. We are currently assessing these genotypes in conjunction with diet and lifestyle factors to more fully understand the disease pathway and its importance in colorectal neoplasia, as interactions between such factors and the respective genotypes also may influence cancer risk.

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