*Short Communication*

# Association of an Exon 1 Polymorphism in the *IGFBP3* Gene with Circulating IGFBP-3 Levels and Colorectal Cancer Risk: The Multiethnic Cohort Study

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## Abstract

**Laboratory and seroepidemiologic studies have suggested that insulin-like growth factor binding protein-3 (IGFBP-3), the main binding protein for IGF-I, may be protective against colorectal cancer. We investigated the association of two polymorphisms (A-202C and G2133C) in the *IGFBP3* gene with plasma IGF hormone levels among 887 randomly selected participants in the Multiethnic Cohort study. We found that these two genetic variants were in strong linkage disequilibrium and were both inversely associated with plasma IGFBP-3. However, the effect on plasma IGFBP-3 levels was stronger for the G2133C variant than the A-202C variant. Thus, we assessed the colorectal cancer risk associated with the G2133C in a case-control study of 817 cases and 1,995 controls nested within the Multiethnic Cohort study. Under the assumption of dominant genetic model, carriers of the *1233C* allele were at 32% increased risk of colorectal cancer [95 % confidence interval (95% CI) for the odds ratio (OR), 1.07-1.62] and that this effect seemed stronger for the rectum (OR for the C allele, 1.95; 95% CI, 1.35-2.83) than the colon (OR, 1.16; 95% CI, 0.92-1.45). These data suggest that the exon 1 G2133C missense variant in *IGFBP3* may be a susceptibility factor for colorectal cancer.** (Cancer Epidemiol Biomarkers Prev 2005;14(5):1319–21)

## Introduction

Plasma levels of insulin-like growth factor binding protein-3 (IGFBP-3), the main binding protein for IGF-I, have been inversely associated with colorectal cancer risk in two nested case-control studies (1, 2). IGFBP-3 may be protective by reducing cell proliferation and inducing apoptosis, independently of decreasing the amount of bioactive IGF-I (3). However, it is not clear whether associations of colorectal cancer with IGF hormones are causal or merely correlates of other aspects of the western lifestyle (e.g., overnutrition and physical inactivity) that increase colorectal cancer risk. In an attempt to confirm the specificity of the relationship between IGFBP-3 and colorectal cancer, we investigated the association of two polymorphisms in the *IGFBP3* gene with IGF hormone levels and assessed the colorectal cancer risk associated with the more promising of the two in a large case-control study nested within the Multiethnic Cohort study.

## Materials and Methods

The design and baseline characteristics of the Multiethnic Cohort have been described in detail elsewhere (4). In short, participants are Hawaii and Los Angeles residents who entered the cohort from 1993 to 1996 by completing a 26-page mail questionnaire about demographic factors, lifestyle (including diet and smoking), medical history, medication use, and family history of common cancer. The cohort included 96,810 men and 118,441 women ages 45 to 75 years at cohort creation in 1993. Twenty-six percent were Japanese American, 23% White, 22% Latino, 16% African American, 7% Native Hawaiian, and 6% other ethnic/racial origin. Colorectal cancer cases were identified through the Rapid Reporting System of the Hawaii Tumor Registry and through quarterly linkages to the Los Angeles County Cancer Surveillance Program, two cancer registries that are members of the Surveillance, Epidemiology and End Results program of the National Cancer Institute. This was complemented by annual linkages to the State of California's cancer registry. All incident colorectal cancer cases occurring since January 1995 in the five main ethnic groups were contacted for inclusion in a case-control study and donation of a blood sample, as were a set of controls, randomly selected from the Multiethnic Cohort participants after stratification by sex and race/ethnicity (5). Samples were collected at the subject's home, processed within 8 hours and stored at 80°C. The participation rate was 74% among cases and 66% among controls.

In an IGF hormone substudy, plasma total IGFBP-3 and IGF-I were measured on a random sample of ~1,000 controls (100 in each sex, race/ethnic group) by ELISA (6). DNA was extracted from blood lymphocytes of the subjects using a standard method (Mini Kit, Qiagen, Valencia, CA). *IGFBP3* genotyping used the method published by Deal et al. (7) for the A-202C promoter variant (rs2854744) and a modification of the protocol of Eggerman et al. (8) for the exon 1 G2133C (G32A) variant (rs2854746). Primers used for the latter variant were as follows: 5′-TGCAGGCGTCATG-CAG-3′ and 5′-CAGCTCCGCGCACAC-3′. This gives a 193-bp

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**Table 1. Geometric means (95% CIs) for plasma IGFBP-3 and IGF-I/IGFBP-3 by *IGFBP3* variant genotypes**

| Genotype | *n* | Plasma, IGFBP-3 (ng/mL) | Molar ratio, IGF-I/IGFBP-3 |
|---|---|---|---|
| G2133C | | | |
| GG | 245 | 2,805 (2,682-2,994) | 0.208 (0.198-0.218) |
| GC | 350 | 2,701 (2,607-2,799) | 0.226 (0.218-0.235) |
| CC | 292 | 2,395 (2,299-2,495) | 0.237 (0.237-0.247) |
| *P* | | <0.0001* | 0.0002 |
| A202C | | | |
| AA | 271 | 2,794 (2,680-2,912) | 0.207 (0.198-0.217) |
| AC | 375 | 2,672 (2,584-2,765) | 0.229 (0.211-0.238) |
| CC | 230 | 2,372 (2,268-2,482) | 0.235 (0.224-0.246) |
| *P* | | <0.0001 | 0.0002 |

NOTE: Adjusted for age at blood draw, sex, and race/ethnicity by covariance analysis.
**P* for a gene-dosage effect variable assigned a value of 1, 2, or 3 according to the number of variant alleles (zero, one, and two alleles, respectively).

PCR product. Because the region is GC rich, 1 mol/L Betaine was added to the PCR reaction. The PCR conditions were an initial denaturation at 94°C for 5 minutes followed by 40 cycles at 94°C for 30 seconds, 64°C for 30 seconds, 72°C for 1 minute with an extension at 72°C for 10 minutes. The PCR product was digested with *Aci*I (New England BioLabs, Beverly, MA) at 37°C overnight. The digests were run on a 4.5% MetaPhor agarose gel. The G allele gives fragments of 85, 28, and 23 bp and many smaller fragments, whereas the C allele gives fragments of 66, 19, 28, and 23 bp plus the smaller fragments. Only the 85- and 66-bp fragments are visible on the gel. The authenticity of this assay was confirmed by DNA sequencing.

In the IGF hormone cross-sectional study, analysis of covariance was used to test for differences in mean plasma levels of IGFBP-3 and the IGF-I/IGFBP-3 molar ratio by genotype, adjusting for sex, race, age, and saturated fat intake, which were determinants of IGFBP-3 levels in this population (6). IGF hormone, genotype, and covariate information was available on 887 subjects for G2133C and 876 subjects for A-202C. The hormone measurements were log transformed to meet the model assumption. Linkage disequilibrium was tested by the $D'$ and $R^2$ statistics. In the nested case-control study, unconditional logistic regression was used to compute odds ratios (OR) and 95% confidence intervals (95% CI) for genotype. All available cases and additional controls were genotyped, resulting in 817 cases and 1995 controls with genotype and covariate information. A gene-dosage effect was tested by inclusion of a trend variable assigned 1, 2, or 3 according to the number of variant alleles present (0, 1, or 2, respectively). The likelihood ratio test was used to test for interaction among certain variables with respect to colorectal cancer. The test compares a main effect, no interaction model with a fully parameterized model containing all possible interaction terms for the variables of interest.

## Results

The subject characteristics have been described earlier for the nested case-control study (5), as well as for the IGF hormone substudy (6). Thirty-four percent of the colorectal cancer cases were Japanese American, 20% African American, 20% Latino, 19% Caucasian, and 7% Native Hawaiian. Fifty-six percent were males and the mean age ± SD at blood draw was 67.2 ± 7.9 years. Seventy-three percent of the cases had colon cancer and 27% rectal cancer.

The two variants genotyped in the IGF hormone substudy showed high linkage disequilibrium with the following *D*s: Whites, 0.96; African Americans, 0.98; Japanese Americans, 0.94; Latinos, 0.91; and Native Hawaiians, 0.92. The corresponding ethnic-specific $R^2$ values were as follows: 0.83, 0.37, 0.89, 0.76, and 0.74, suggesting that recombination may have occurred in African Americans.

Table 1 presents the relationships between genotype and plasma hormone levels in the IGF hormone substudy. For both polymorphisms, the variant alleles were associated with lower mean levels for plasma IGFBP-3 and the molar ratio IGF-I/IGFBP-3. These data are consistent with previous findings for the A-202C variant (7) and are consistent with the variants possibly increasing risk of colorectal cancer.

Next, we simultaneously introduced both variants into the multiple regression of genotype on plasma IGFBP-3 levels and found that only the *P* for the G2133C variant remained of borderline statistical significance ($P$ = 0.07), whereas the −202C variant showed no significance ($P$ = 0.79). We interpreted this finding as suggesting that the effect of the exon 1 missense variant on hormone levels was greater than that of the −202C variant and that, consequently, the former was the stronger candidate for disease association. Thus, only the G2133C variant was genotyped in cases and additional controls for the nested case-control study.

Based on the controls, the frequency for the *2133C* allele was 0.22 for Japanese, 0.56 in Caucasians, 0.66 in African Americans, 0.62 in Latinos, and 0.46 in Native Hawaiians. The genotype distributions were consistent with Hardy-Weinberg equilibrium in each ethnic group, except Latinos ($P$ = 0.02) and Hawaiians ($P$ = 0.01).

Table 2 shows the ORs for colorectal cancer by genotype for the G2133C polymorphism. A statistically significant association was found with a dominant genetic model for the C allele ($P$ = 0.008). The age-, sex-, and race/ethnicity-adjusted OR for the presence of the C allele was 1.32 (95% CI, 1.07-1.62). This effect was only slightly modified by further adjustment for other covariates (Table 2) or after exclusion of Latinos and Hawaiians (OR, 1.29; 95% CI, 1.01-1.15). The ORs for the C allele among Japanese, Caucasian, Hawaiian, African American, and Latino subjects were 1.34 (95% CI, 0.98-1.83), 0.97 (95% CI, 0.61-1.52), 1.42 (95% CI, 0.75-2.70), 1.69 (95% CI, 0.86-3.30), and 1.37 (95% CI, 0.81-2.31), respectively. The effect of the C allele was similar in both sexes and for early-stage (*in situ*/localized) and late-stage (regional/distant) tumors (data not shown). However, the effect seemed greater for the rectum (OR, 1.95; 95% CI, 1.35-2.83) than the colon (OR, 1.16; 95% CI, 0.92-1.45). No interaction was suggested between *IGFBP3* and body mass index or height.

**Table 2. Colorectal cancer ORs (95% CIs) for the *IGFBP3* G2133C variant**

| Genotype | Model 1* | | Model 2† | |
|---|---|---|---|---|
| | *n* | OR (95% CI) | *n* | OR (95% CI) |
| GG | 235/585 | 1.00 | 205/537 | 1.00 |
| GC | 355/805 | 1.35 (1.09-1.69) | 302/739 | 1.27 (1.01-1.60) |
| CC | 229/605 | 1.25 (0.98-1.61) | 193/526 | 1.22 (0.93-1.60) |
| | | *P* = 0.088‡ | | *P* = 0.161 |
| Any C | | 1.32 (1.07-1.62) | | 1.26 (1.00-1.57) |
| | | *P* = 0.008§ | | *P* = 0.045 |

*Adjusted for age at blood draw, sex, and race/ethnicity by unconditional logistic regression.
†Further adjusted for pack-years of cigarette smoking, body mass index, hours per week in vigorous activity, and fiber and ethanol intakes. Numbers of subjects differ due to missing covariates.
‡*P* for a gene-dosage effect variable assigned a value of 1, 2, or 3 according to the number of C alleles (zero, one, and two C alleles, respectively).
§*P* for dominant genetic effect.

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## Discussion

In this study, we found that the *IGFBP3* 2133C variant allele was associated with lower circulating IGFBP-3 hormone levels and an increased risk of colorectal cancer. This increased risk was suggested in four of the five ethnic groups studied and seemed stronger for rectal cancer.

IGFBP-3 binds 70% to 80% of IGF-I and thus limits availability of this crucial growth factor to cells. IGFBP-3 also exhibits independent antiproliferative and proapoptotic activities (3) and has been shown to inhibit the development of colon tumors in experimental animals (9). Two nested case-control studies observed a marked reduction in colorectal cancer risk with plasma IGFBP-3 (1, 2). However, these findings were not confirmed in subsequent studies that used a different type of ELISA assay to measure plasma IGFBP-3. Indeed, these studies observed a positive association with colorectal cancer (10-12). A recent meta-analysis of these published data estimated the colorectal cancer OR comparing the 75th to the 25th percentile of circulating IGFBP-3 to be 0.77 (95% CI, 0.36-1.66; ref. 13). Thus, the data on IGFBP-3 levels and colorectal cancer remain inconsistent. The independent association of colorectal cancer with inherited genetic variants that affect IGFBP-3 levels, presumably over the lifetime, may clarify this relationship.

In agreement with our data, the A-202C transition polymorphism has previously been associated with lower plasma levels of IGFBP-3 (6, 14); it was not associated with colorectal cancer or breast cancer in recent studies (14, 15). In addition, we found that the exon 1 G32A missense variant was in strong linkage disequilibrium with the −202C variant, was more strongly related to plasma IGFBP-3 levels, and was associated with a 30% increase in colorectal cancer risk. Confirmation of these findings and a systematic characterization of the association between colorectal cancer and SNPs and haplotypes recently identified in this gene are warranted.

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