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Genetic Determinants of Circulating Insulin-Like Growth Factor (IGF)-I, IGF Binding Protein (BP)-1, and IGFBP-3 Levels in a Multiethnic Population

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Context: Both circulating levels and genetic variation of IGFs have been associated with cancer risk, yet the relationship between the two is not well understood.

Objective: To investigate whether common genetic variation in *IGF1*, *IGF binding protein 1* (*IGFBP1*), and *IGFBP3* influences circulating levels of IGF-I, IGFBP-1, and IGFBP-3, we conducted a cross-sectional study of African-American, Native Hawaiian, Japanese-American, Latino, and white men and women in the Multiethnic Cohort.

Design: Plasma levels of IGF-I, IGFBP-1, and IGBFP-3 were measured by ELISA in a random sample of 837 Multiethnic Cohort participants. Previously identified tag single nucleotide polymorphisms (SNPs) for *IGF1* (29 tag SNPs) and *IGFBP1*/*IGFBP3* (23 tag SNPs) were genotyped among the 837 participants. Analysis of covariance was conducted to test for differences in mean IGF-I, IGFBP-1, and IGFBP-3 levels across respective *IGF1*, *IGFBP1,* and *IGFBP3* genotypes, adjusting for previously identified dietary and lifestyle correlates.

Results: Five highly correlated *IGFBP3* SNPs (rs3110697, rs2854747, rs2854746, rs2854744, and rs2132570) demonstrated strongly significant associations with IGFBP-3 levels when conservatively adjusted for multiple hypothesis testing (Bonferroni adjusted P trends $= 7.75 \times 10^{-8}$ to 1.44×10^{-5}). Patterns of associations were consistent across the five racial/ethnic groups.

Conclusion: In summary, our study suggests that common genetic variation in *IGFBP3* influences circulating levels of IGFBP-3 among African-Americans, Native Hawaiians, Japanese-Americans, Latinos, and whites. **(*J Clin Endocrinol Metab* 92: 3660–3666, 2007)**

IGFs PLAY A KEY ROLE in regulating cellular growth. Circulating levels of IGF-I, IGF binding protein (IGFBP)-1, and IGFBP-3 have been associated with increased risk of breast, colorectal, and prostate cancers (1–3). More recently, genetic polymorphisms within *IGF1*, *IGFBP1*, and *IGFBP3* have been investigated for their influence on cancer susceptibility (2, 4–6). However, the relationship between circulating levels of IGF-I, IGFBP-1, and IGFBP-3 and their genetic polymorphisms has yet to be clearly defined.

Twin studies have estimated that approximately 40–60% of the interindividual variation in circulating levels of IGF-I, IGFBP-1, and IGFBP-3 are attributed to heritable factors (7, 8). Previous studies have focused primarily on a small number of polymorphisms, such as the *IGF1* $(CA)_n$ repeat polymorphism (9–11) and the *IGFBP3* A-202C (rs2854744) polymorphism (4, 11–18), whereas a recent study investigated *IGF1*, *IGFBP1,* and *IGFBP3* tagging polymorphisms (4). From these studies, the most consistently observed association is the lower levels of circulating IGFBP-3 in the presence of the C allele of the *IGFBP3* A-202C promoter polymorphism (4, 11–18). The great majority of these previous studies have been limited to whites (4, 12–16, 18), and whether this association exists in other racial/ethnic groups is a question that has yet to be explored fully.

Within the Multiethnic Cohort (MEC), we have recently demonstrated that common genetic variation in *IGF1* influences prostate cancer susceptibility (6). Furthermore, we have previously identified dietary and lifestyle regulators of circulating levels of IGF-I, IGFBP-1, and IGFBP-3 (19–21). These studies have identified racial/ethnic differences in circulating levels of IGF-I and IGFBP-3, as well as the interactive effects between race/ethnicity and obesity on IGF-I levels (19, 20). Building on this prior work, we investigated within the MEC whether inherited variation in *IGF1*, *IGFBP1*, and *IGFBP3* influences circulating levels of IGF-I, IGFBP-1, and IGFBP-3. This is the first multiethnic study to assess comprehensively the genetic diversity of these loci in relation to their circulating levels.

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Abbreviations: BMI, Body mass index; IGFBP, IGF binding protein; MEC, Multiethnic Cohort; SNP, single nucleotide polymorphism.

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Subjects and Methods

MEC

The MEC study is a large population-based cohort study of more than 215,000 men and women from Hawaii and Los Angeles. The cohort is comprised predominantly of five racial/ethnic groups: African-Americans, Native Hawaiians, Japanese-Americans, Latinos, and whites. Participants between the ages of 45 and 75 yr were recruited from 1993–1996, and completed a 26-page self-administered questionnaire that included information regarding height, weight, medical history, family history, diet, dietary supplements and medication use, and physical activity. All participants were between the ages of 47 and 82 yr at the time of blood draw. Further details are provided elsewhere (22).

The blood samples used in this study were collected on a subcohort of about 5000 randomly selected participants. The draw was completed in the morning, typically at the person's home, after informed consent was obtained. The participation rate for providing a blood sample was 66%. This study was approved by the institutional review boards of the University of Hawaii and University of Southern California.

Plasma levels of IGF-I, IGFBP-1, and IGFBP-3 were measured among a random sample of 1000 of these MEC control participants [100 subjects in each sex and racial/ethnic group with equal representation of each 5-yr age group at blood draw (>45 yr for men and >55 yr for women)] (19–21). A total of 959 subjects had complete plasma measurements of IGFs, of whom 133 subjects were excluded for having prevalent breast, prostate, or colon cancer, or were premenopausal or taking estrogen replacement therapy at the time of blood draw, or had incomplete body mass index (BMI) information. A total of 826 subjects were included in this analysis.

Plasma measurement

To reduce interbatch variation, we blinded laboratory personnel to the sex and ethnicity of samples, and included an equal number of subjects from each sex/ethnic group for each assay batch. Plasma levels of IGF proteins were measured by ELISAs from Diagnostic System Laboratories (Webster, TX). IGF-I assays included an acid-ethanol precipitation of IGFBPs to minimize the interference of IGFBPs. The overall average intrabatch coefficient of variation was less than 10% for all IGF-related proteins (19–21). The overall average interbatch coefficients of variation were 13.9%, 14.6%, and 10.4% for IGF-I, IGFBP-1, and IGFBP-3, respectively (19–21).

Tag single nucleotide polymorphism (SNP) selection

For *IGF1*, we previously selected 29 tag SNPs to capture the common genetic variation of 64 SNPs (minor allele frequency ≥ 5%) that were genotyped in a multiethnic panel of 349 controls, spanning 156 kb at a density of one SNP every 2.4 kb (the results can be found in supplemental Table 1, which is published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) (6). The proportion of *IGF1* SNPs that were captured at a pairwise $r^2 > 0.8$ was 75% for African-Americans, 98% for Native Hawaiians, 96% for Japanese-Americans, 88% for Latinos, and 90% for whites. For

TABLE 1. Study characteristics of 826 MEC participants used for IGF analyses

	African-Americans	Native Hawaiians	Japanese-Americans	Latinos	Whites
n (%)	150 (18.2)	161 (19.5)	170 (20.6)	180 (21.8)	165 (20.0)
Mean age ± SD (yr)	65.9 ± 8.2	65.4 ± 7.7	66.9 ± 8.2	65.9 ± 8.4	66.1 ± 8.3
No. of males (%)	73 (48.7)	98 (60.9)	92 (54.1)	90 (50.0)	92 (55.8)
BMI (kg/m^2)					
<25, n (%)	35 (23.3)	44 (27.3)	102 (60.0)	43 (23.9)	57 (34.6)
25–29, n (%)	62 (41.3)	61 (37.9)	56 (32.9)	89 (49.4)	75 (44.9)
≥30, n (%)	53 (35.3)	56 (34.8)	12 (7.1)	48 (26.7)	34 (20.6)

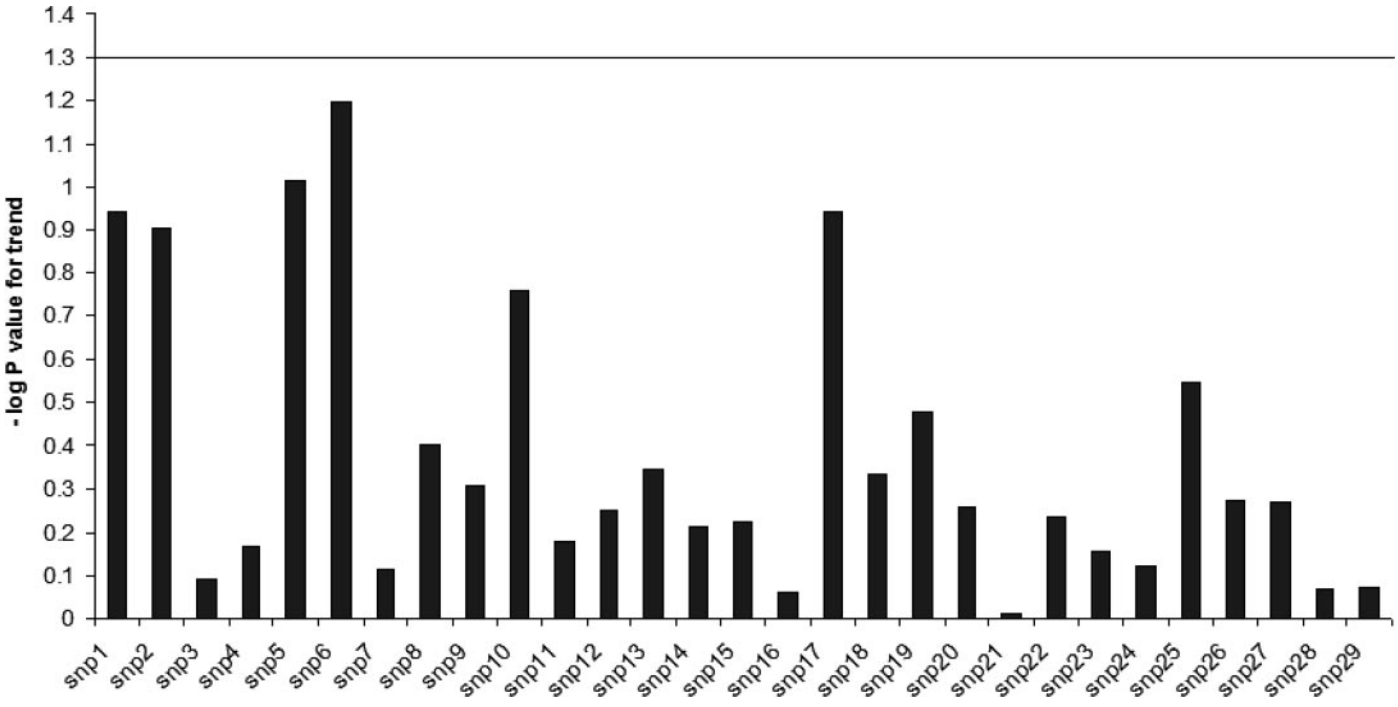

FIG. 1. Association between 29 *IGF1* tag SNPs and circulating IGF-I levels. *Horizontal line* indicates $P = 0.05$.

IGFBP1 and *IGFBP3*, we previously identified 23 tag SNPs to capture the common genetic variation of 36 SNPs (minor allele frequency ≥ 5%) that were genotyped in a multiethnic panel, spanning the 71-kb locus at a density of one SNP every 2.0 kb (the results can be found in supplemental Table 2, which is published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org) (5). The *IGFBP3* missense polymorphism (rs2854746) and the A-202C polymorphism (rs2854744) were "forced" in to be selected as tags to ensure that these potentially relevant SNPs were examined. The proportion of *IGFBP1* and *IGFBP3* SNPs that were captured at a pairwise $r^2 > 0.8$ was 76% for African-Americans, 84% for Native Hawaiians, 89% for Japanese-Americans, 86% for Latinos, and 91% for whites.

Genotyping

IGF1, *IGFBP1*, and *IGFBP3* tag SNPs were genotyped using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA) by the University of Southern California/Norris Cancer Genomics Core Facility (5, 6). We tested for Hardy-Weinberg equilibrium for each SNP among controls of each racial/ethnic group. All SNPs were in Hardy-Weinberg equilibrium (at $P > 0.01$ level). For *IGF1*, the concordance for replicate samples was 99.7%, and the average successful genotyping was 97.9%. For *IGFBP1/IGFBP3*, the concordance for replicate samples was 99.8%, and the average successful genotyping was 97.4%.

Statistical analysis

We conducted an analysis of covariance to test for differences in mean IGF-I, IGFBP-1, and IGFBP-3 levels across respective *IGF1*, *IGFBP1*, and *IGFBP3* genotypes/haplotypes. Haplotype frequencies were estimated by the expectation-maximization algorithm using the tagSNP software (23). Haplotype dosage (*i.e.* an estimate of the number of copies of haplotype h) for each individual and each haplotype, h, was computed using that individual's genotype data and haplotype frequency estimates obtained from the E-M algorithm (24). Statistical analyses were performed on logarithmically transformed values of IGF-I, IGFBP-1, and IGFBP-3. Our multivariate regression analyses were based on the previously described models reported by DeLellis Henderson *et al.* (19–21). For IGF-I levels, our model included age, racial/ethnic group, sex, BMI, an interaction term for racial/ethnic group and BMI, and genotype/haplotype. For IGFBP-1 levels, our model included age, racial/ethnic group, sex, BMI, regular soda intake, an interaction term for age and BMI, and genotype/haplotype. For IGFBP-3 levels, our model included age, racial/ethnic group, sex, BMI, fat from meat intake, and genotype/haplotype. We calculated the partial correlation between genotype/haplotype and respective IGF levels, controlling for aforementioned covariates to determine the contribution of genotype/haplotype to the variance in IGF levels. We tested for heterogeneity of genotype effects across racial/ethnic groups by including an interaction term between genotype and racial/ethnic group in a multivariable model. We used the r^2 selection method in conjunction with Mallow's Cp to evaluate which combination of genotypes provided the best fit of our model of IGF levels. All analyses were performed in SAS version 9.0 (SAS Institute Inc., Cary, NC).

Results

Selected characteristics of the 826 MEC participants used in this study are presented in Table 1. Approximately 18–22% of the study subjects were from each of the five racial/ethnic groups. The mean age (~66 yr) was similar across the five groups. As previously reported, BMI varied markedly across the five racial/ethnic groups. African-Americans had the highest proportion of BMI more than 25 kg/m^2, followed by Latinos, Native Hawaiians, whites, and Japanese-Americans.

For IGF-I, there were no significant associations between the 29 *IGF1* tag SNPs and circulating levels of IGF-I (*P* trends >0.06; Fig. 1). For IGFBP-1 and IGFBP-3, seven of the 23 *IGFBP1/IGFBP3* tag SNPs were nominally statistically significantly associated with IGFBP-1 and IGFBP-3 levels (*P* trends = 3.37×10^{-9} to 0.047; Fig. 2). In addition, four *IGF1* SNPs [SNP11 (rs10735380), SNP25 (rs2139570), SNP27 (rs4764695), and SNP28 (rs1520219)] and one *IGFBP3* SNP [SNP16 (rs2453839)] displayed evidence of heterogeneity across racial/ethnic groups on IGF levels. Results stratified by racial/ethnic groups for these five SNPs are presented in supplemental Table 3 (published as supplemental data on The Endocrine Society's Journals Online web site at http://jcem.endojournals.org).

The most compelling associations were seen in *IGFBP3*, in which five SNPs were strongly associated with circulating IGFBP-3 levels: SNP17 (rs3110697), SNP19 (rs2854747), SNP20 (rs2854746), SNP21 (rs2854744), and SNP22 (rs2132570) (*P* trends = 3.37×10^{-9} to 6.24×10^{-7}) (Table 2). We applied a Bonferroni correction of 23 *IGFBP1/IGFBP3*

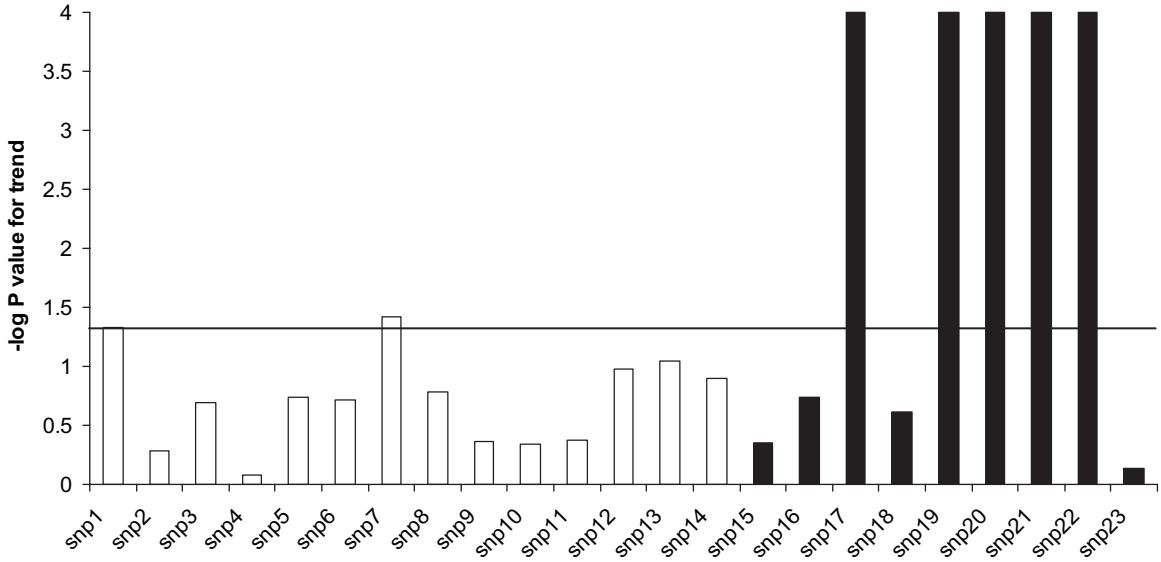

FIG. 2. Association between 23 *IGFBP1* and *IGFBP3* tag SNPs and circulating IGFBP-1 and IGFBP-3 levels. *White and black bars* indicate *IGFBP1* and *IGFBP3* SNPs, respectively. *Horizontal line* indicates $P = 0.05$. *P* values for SNPs 17, 19, 20, 21, 22, and 23 < 0.0001.

SNP tests to the *P* values of these five SNPs, and the adjusted *P* values remained statistically significant (*P* trends = 7.75×10^{-8} to 1.44×10^{-5}). Geometric mean circulating levels of IGFBP-3 decreased with additional copies of the minor allele for four *IGFBP3* polymorphisms (SNP17, SNP19, SNP21, and SNP22), while IGFBP-3 levels increased with additional copies of the minor allele for SNP20. For all five racial/ethnic groups, these five *IGFBP3* polymorphisms displayed relatively consistent patterns of associations on IGFBP-3 levels (Table 3).

The five associated *IGFBP3* polymorphisms (SNP17, SNP19, SNP20, SNP21, and SNP22) were reasonably highly correlated with each other across the five racial/ethnic groups (Table 4), with the exception of SNP22 (rs2132570) among African-Americans (r^2 = 0.07–0.18). Of these five polymorphisms, SNP20 (rs2854746) and SNP22 (rs2132570) together provided the best fit for our model of IGFBP-3 levels using the r^2 selection method in conjunction with Mallow's Cp. SNP20 and SNP22 explained approximately 1% of the overall variance in IGFBP-3 levels, with our full model capturing 11.5% of variance in IGFBP-3 levels.

The *IGFBP3* locus is located within a region of strong linkage disequilibrium from SNP17 to SNP23 (5). Two common haplotypes within this region, 3A (total frequency 44%) and 3B (total frequency 19%) (5), were significantly associated with circulating IGFBP-3 levels ($P = 3.83 \times 10^{-5}$ and $P = 1.76 \times 10^{-5}$, respectively; data not shown). Haplotype 3A was associated with higher levels of IGFBP-3 with increasing number of haplotypes. In contrast, haplotype 3B was associated with lower levels of IGFBP-3 with increasing number of haplotypes. These two haplotypes differed only at the alleles of the five *IGFBP3*-associated SNPs (SNP17, 19, 20, 21, and 22). Haplotype 3A harbored the alleles of these five polymorphisms that were associated with higher IGFBP-3 levels, whereas haplotype 3B harbored the alleles associated

TABLE 2. Geometric means for plasma IGFBP-3 levels (ng/ml) by genotype for the five associated *IGFBP3* SNPs

		All	
		n	Geometric mean (95% CI)[a]
SNP17	GG	284	2749.51 (2640.04, 2863.52)
rs3110697	GA	347	2647.85 (2554.60, 2744.50)
	AA	175	2343.20 (2223.60, 2469.24)
	P trend		4.83×10^{-9}
SNP19	AA	291	2743.02 (2635.04, 2855.43)
rs2854747	AG	342	2659.70 (2565.40, 2757.47)
	GG	172	2326.08 (2205.86, 2452.85)
	P trend		3.37×10^{-9}
SNP20	GG	259	2415.25 (2311.13, 2524.07)
rs2854746	GC	329	2644.32 (2548.84, 2743.37)
	CC	211	2801.95 (2667.28, 2943.41)
	P trend		1.24×10^{-9}
SNP21	AA	239	2729.96 (2609.67, 2855.80)
rs2854744[b]	AC	356	2665.86 (2571.91, 2763.23)
	CC	209	2384.72 (2271.44, 2503.65)
	P trend		6.24×10^{-7}
SNP22	GG	477	2714.55 (2631.64, 2800.08)
rs2132570	GT	270	2542.76 (2440.00, 2649.85)
	TT	57	2251.47 (2054.75, 2467.02)
	P trend		1.56×10^{-7}

CI, Confidence interval.
[a] Adjusted for age, ethnicity, sex, BMI, and fat from meat intake.
[b] *IGFBP3* A-202C.

TABLE 3. Geometric means for plasma IGFBP-3 levels (ng/ml) for the five associated *IGFBP3* SNPs by racial/ethnic group

		African-Americans		Native Hawaiians		Japanese-Americans		Latinos		Whites	
		n	Geometric mean (95% CI)[a]	n	Geometric mean (95% CI)[a]	n	Geometric mean (95% CI)[a]	n	Geometric mean (95% CI)[a]	n	Geometric mean (95% CI)[a]
SNP17	GG	60	2614.94 (2414.23, 2832.34)	57	2773.95 (2552.39, 3014.75)	93	2781.75 (2541.93, 3044.2)	25	2544.82 (2194.98, 2950.42)	49	2834.26 (2580.16, 3113.38)
rs3110697	GA	63	2658.23 (2460.28, 2872.11)	66	2886.96 (2669.2, 3122.48)	62	2450.6 (2210.03, 2717.35)	84	2469.02 (2284.55, 2668.38)	72	2731.17 (2520.03, 2960.01)
	AA	25	2104.91 (1846.59, 2399.37)	31	2626.58 (2343.53, 2943.82)	14	2191.51 (1783.3, 2693.16)	68	2069.86 (1892.3, 2264.09)	37	2667.4 (2395.71, 2969.9)
	P trend		0.036		0.604		0.013		0.003		0.391
SNP19	AA	61	2567.67 (2374.51, 2776.55)	58	2807.47 (2582.21, 3052.38)	96	2746.78 (2514.36, 3000.67)	24	2593.28 (2229.83, 3015.97)	52	2822.24 (2578.42, 3089.12)
rs2854747	AG	62	2719.46 (2518.32, 2936.66)	67	2832.8 (2619.88, 3063.01)	60	2479.48 (2226.29, 2761.46)	85	2455.07 (2272.48, 2652.32)	68	2759.05 (2543, 2993.45)
	GG	24	2060.23 (1803.54, 2353.45)	31	2630.13 (2342.59, 2952.96)	12	2107.85 (1689.04, 2630.5)	68	2054 (1876.67, 2248.1)	37	2648.45 (2380.82, 2946.17)
	P trend		0.072		0.453		0.008		0.001		0.371
SNP20	GG	60	2313.38 (2131.29, 2511.02)	36	2601.58 (2340.26, 2892.08)	12	2105.21 (1686.17, 2628.39)	90	2200.48 (2033.21, 2381.51)	61	2658.83 (2440, 2897.27)
rs2854746	GC	69	2705.47 (2510.78, 2915.26)	63	2834.27 (2614.03, 3073.08)	59	2443.5 (2191.18, 2724.88)	71	2371.23 (2176, 2583.97)	67	2787.29 (2566.21, 3027.42)
	CC	17	2615.99 (2258.98, 3029.43)	53	2809.95 (2575.11, 3066.21)	95	2770.86 (2535.35, 3028.24)	16	2801.59 (2337.77, 3357.43)	30	2824.46 (2508.15, 3180.66)
	P trend		0.025		0.315		0.003		0.017		0.363
SNP21	AA	49	2481.61 (2269.35, 2713.73)	50	2826.2 (2586.52, 3088.1)	90	2743.37 (2506.06, 3003.14)	17	2715.66 (2276.19, 3239.98)	33	2794.26 (2493.12, 3131.79)
rs2854744[b]	AC	74	2706.03 (2513.66, 2913.13)	68	2833.97 (2615.6, 3070.57)	61	2469.33 (2215.37, 2752.4)	78	2475.76 (2282.59, 2685.27)	75	2786.41 (2571.3, 3019.51)
	CC	26	2290.29 (2026.66, 2588.21)	37	2607.22 (2352.77, 2889.19)	12	2109.93 (1686.83, 2639.14)	81	2100.08 (1933.1, 2281.47)	53	2638.95 (2408.69, 2891.22)
	P trend		0.601		0.275		0.009		0.001		0.387
SNP22	GG	116	2571.67 (2424.1, 2728.22)	100	2782.61 (2604.86, 2972.48)	99	2746.25 (2515.87, 2997.72)	66	2510.97 (2292.39, 2750.38)	96	2827.04 (2651.05, 3014.71)
rs2132570	GT	32	2450.49 (2183.29, 2750.38)	46	2677.49 (2436.34, 2942.5)	59	2472.25 (2218.84, 2754.6)	79	2354.61 (2168.95, 2556.15)	54	2760.24 (2524.33, 3018.19)
	TT	1	3537.44 (1888.7, 6625.46)	7	3045.26 (2394.66, 3872.63)	11	2086.43 (1659.67, 2622.93)	35	1943.83 (1711.13, 2208.17)	5	2707.13 (2049.32, 3576.09)
	P trend		0.448		0.448		0.025		0.006		0.885

CI, Confidence interval.
[a] Adjusted for age, sex, BMI, and fat from meat intake.
[b] *IGFBP3* A-202C.

TABLE 4. Pairwise correlation (D′ and r^2) between the *IGFBP3* SNPs associated with plasma IGFBP-3 levels by racial/ethnic group

	SNP17 rs3110697	SNP19 rs2854747	SNP20 rs2854746	SNP21 rs2854744	SNP22 rs2132570
African-Americans					
rs3110697		0.97	1.00	0.93	0.68
rs2854747	0.85		1.00	0.96	0.51
rs2854746	0.25	0.23		1.00	1.00
rs2854744	0.69	0.67	0.31		0.87
rs2132570	0.14	0.09	0.07	0.18	
Native Hawaiians					
rs3110697		1.00	0.92	0.93	1.00
rs2854747	0.94		1.00	1.00	1.00
rs2854746	0.59	0.65		1.00	1.00
rs2854744	0.67	0.73	0.89		1.00
rs2132570	0.56	0.60	0.38	0.43	
Japanese-Americans					
rs3110697		1.00	1.00	1.00	1.00
rs2854747	0.96		1.00	1.00	1.00
rs2854746	0.96	1.00		1.00	1.00
rs2854744	0.96	1.00	1.00		1.00
rs2132570	0.95	1.00	1.00	1.00	
Latinos					
rs3110697		1.00	1.00	0.96	1.00
rs2854747	1.00		1.00	0.96	1.00
rs2854746	0.69	0.69		1.00	1.00
rs2854744	0.76	0.76	0.84		1.00
rs2132570	0.45	0.45	0.30	0.36	
Whites					
rs3110697		0.94	0.93	0.95	0.81
rs2854747	0.86		1.00	1.00	0.94
rs2854746	0.35	0.41		1.00	1.00
rs2854744	0.45	0.51	0.81		1.00
rs2132570	0.29	0.38	0.18	0.22	

The upper matrix represents D′ values among SNPs. The lower matrix represents r^2 values among SNPs.

with lower IGFBP-3 levels. These *IGFBP3* haplotypes explained approximately 1% and the full model 12% of the overall variance in IGFBP-3 levels.

Discussion

In this multiethnic study of African-Americans, Native Hawaiians, Japanese-Americans, Latinos, and whites, we comprehensively examined the genetic diversity in *IGFI*, *IGFBP1*, and *IGFBP3*, and tested whether common genetic variation at these loci influences circulating levels of IGF-I, IGFBP-1, and IGFBP-3. Our results indicate that inherited variation in *IGFBP3* was associated with circulating levels of IGFBP-3. Specifically, we identified five *IGFBP3* polymorphisms that were consistently associated with circulating IGFBP-3 levels across the five racial/ethnic groups. In addition, when we corrected for multiple hypotheses testing using a conservative Bonferroni approach, these *IGFBP3* polymorphisms remained highly significant.

A previous study from the United Kingdom similarly examined *IGF1* and *IGFBP3* tagging polymorphisms in relation to circulating IGF-I and IGFBP-3 levels (4). Of nine *IGF1* polymorphisms tested in that study, five and two polymorphisms were associated with circulating levels of IGF-I among females and males, respectively (4). We examined two of the *IGF1* polymorphisms [rs1520220 (SNP18) and rs2946834 (SNP21)] that were associated with IGF-I levels in the United Kingdom study ($P = 0.003$ and $P = 0.02$, respectively) and found no association ($P = 0.55$ and $P = 0.85$, respectively). This discrepancy may be due to our reduced power for white-specific analysis, having 168 whites in contrast to 937 European subjects in the United Kingdom study. Of four *IGFBP3* polymorphisms tested in the United Kingdom study, three and two polymorphisms were associated with circulating levels of IGFBP-3 among females and males, respectively (4). We examined the A-202C polymorphism that was the most strongly associated with IGFBP-3 levels in the United Kingdom study ($P < 10^{-9}$ for females; $P = 0.00004$ for males) and found similar highly significant effects ($P = 6.24 \times 10^{-7}$). In total, nine studies, including our current study, have examined the *IGFBP3* A-202C polymorphism in relation to IGFBP-3 levels and have consistently reported significant effects (4, 11–16, 18). In a study from the European Prospective Investigation Cohort, seven *IGFBP1* polymorphisms were tested for their association with circulating IGFBP-1 levels, and no significant associations were observed (18). The two nominally associated *IGFBP1* polymorphisms (rs10228265 and rs1065781) in our study have not previously been examined.

Because of the strong regional correlation across the *IGFBP3* locus (5), the predisposing allele responsible for influencing circulating levels of IGFBP-3 remains to be identified. The *IGFBP3* A-202C polymorphism is a promising candidate because it was originally shown by Deal *et al.* (12) to influence promoter activity. The A allele has been shown in an *in vitro* assay to have a higher promoter activity, compared with the C allele. This is in line with the lower levels of IGFBP-3 observed in the presence of the C allele. In light of the strong biological support for this polymorphism, coupled with the consistent evidence of this association from prior reports and our multiethnic study, future work should

examine the relationship between this polymorphism and tissue expression to assess further its functional role.

For four of the five *IGFBP3* polymorphisms that were strongly associated with circulating levels of IGFBP-3, lower levels were observed in the presence of the minor allele. Because IGFBP-3 is the principal binding protein of circulating IGF-I, binding more than 90% of IGF-I in conjunction with the acid-labile subunit (25), lower levels of IGFBP-3 due to genetic variation may increase the bioavailability of IGF-I. This may ultimately influence the bioactivity of IGF-I in the circulation and tissues, leading to cellular growth and cancer susceptibility.

In previous nested case-control studies within the MEC, there was no association between common genetic variation in *IGFBP3* and breast and prostate cancer risk (5). The significant effect of the five *IGFBP3* polymorphisms on circulating IGFBP-3 levels in the absence of an effect on cancer highlights the complexity of the hormonal milieu of the IGF system. It is possible that other genetic variants and environmental factors may act individually or in concert to modulate the exposure of target tissues to IGFs and cancer susceptibility. As well, it is possible that *IGFBP3* variants may influence hormonal levels, but not cancer risk. This has been seen for *CYP19*, in which genetic variation at this locus predicts estrogen levels, but not breast cancer risk (26).

Our study has several limitations. Although we were able to capture the majority of the common genetic variation across the *IGF1*, *IGFBP1*, and *IGFBP3* genes, we have not exhaustively captured all of the common genetic diversity of these loci among the five racial/ethnic groups, especially among African-Americans (see *Tag single nucleotide polymorphism (SNP) selection*). In addition, our study cannot exclude the possibility that rare genetic variants may influence circulating levels.

In conclusion, our study of African-Americans, Native Hawaiians, Japanese-Americans, Latinos, and whites suggests that common genetic variation in *IGFBP3* influences circulating IGFBP-3 levels, beyond the effects of previously reported dietary and lifestyle correlates. With replication in larger cohorts such as the National Cancer Institute Breast and Prostate Cancer Cohort Consortium (http://epi.grants.cancer.gov/BPC3/), additional fine-mapping and mechanistic work will be needed to pinpoint the causal variant. Furthermore, it remains to be determined whether other genes in the GH (17) and IGF family impact circulating levels of IGFs because it may be the cumulative effect of several genes that drives cancer predisposition.

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