Short Communication

Haplotype-Based Association Studies of *IGFBP1* and *IGFBP3* with Prostate and Breast Cancer Risk: The Multiethnic Cohort

Iona Cheng,[1] Kathryn L. Penney,[2,3,4,6] Daniel O. Stram,[9] Loic Le Marchand,[10] Elena Giorgi,[9] Christopher A. Haiman,[9] Laurence N. Kolonel,[10] Malcolm Pike,[9] Joel Hirschhorn,[2,3,5,7] Brian E. Henderson,[9] and Matthew L. Freedman[2,4,8]

[1]Department of Epidemiology and Biostatistics and Center for Human Genetics, University of California-San Francisco, San Francisco, California; [2]Program in Medical and Population Genetics, Broad Institute of Harvard and Massachusetts Institute of Technology, Cambridge, Massachusetts; Departments of [3]Genetics, [4]Medicine, and [5]Pediatrics, Harvard Medical School; [6]Department of Molecular Biology, Massachusetts General Hospital; [7]Division of Genetics and Endocrinology, Children's Hospital and Department of Pediatrics; [8]Dana-Farber Cancer Institute, Boston, Massachusetts; [9]Department of Preventive Medicine, Norris Comprehensive Cancer Center, Keck School of Medicine, University of Southern California, Los Angeles, California; and [10]Cancer Epidemiology Program, Cancer Research Center of Hawaii, University of Hawaii, Honolulu, Hawaii

Abstract

Collective evidence suggests that the insulin-like growth factor (IGF) system plays a role in prostate and breast cancer risk. IGF-binding proteins (IGFBP) are the principal regulatory molecules that modulate IGF-I bioavailability in the circulation and tissues. To examine whether inherited differences in the *IGFBP1* and *IGFBP3* genes influence prostate and breast cancer susceptibility, we conducted two large population-based association studies of African Americans, Native Hawaiians, Japanese Americans, Latinos, and Whites. To thoroughly assess the genetic variation across the two loci, we (*a*) sequenced the *IGFBP1* and *IGFBP3* exons in 95 aggressive prostate and 95 advanced breast cancer cases to ensure that we had identified all common missense variants and (*b*) characterized the linkage disequilibrium patterns and common haplotypes by genotyping 36 single nucleotide polymorphisms (SNP) spanning 71 kb across the loci (~20 kb upstream and ~40 kb downstream, respectively) in a panel of 349 control subjects of the five racial/ethnic groups. No new missense SNPs were found. We identified three regions of strong linkage disequilibrium and selected a subset of 23 tagging SNPs that could accurately predict both the common *IGFBP1* and *IGFBP3* haplotypes and the remaining 13 SNPs. We tested the association between *IGFBP1* and *IGFBP3* genotypes and haplotypes for their associations with prostate and breast cancer risk in two large case-control studies nested within the Multiethnic Cohort [prostate cases/controls = 2,320/2,290; breast cases (largely postmenopausal)/controls = 1,615/1,962]. We observed no strong associations between *IGFBP1* and *IGFBP3* genotypes or haplotypes with either prostate or breast cancer risk. Our results suggest that common genetic variation in the *IGFBP1* and *IGFBP3* genes do not substantially influence prostate and breast cancer susceptibility. (Cancer Epidemiol Biomarkers Prev 2006;15(10):1993–7)

Introduction

Experimental and epidemiologic studies provide strong support for the role of the insulin-like growth factor (IGF) system in prostate and breast cancer tumorigenesis (1-3). IGF-I is a mitogen that regulates cell proliferation and also inhibits apoptosis (4). The bioavailability of IGF-I in the circulation and tissues is tightly regulated by IGF-binding proteins (IGFBP) that bind IGF-I with high affinity and specificity (5). In the circulation, >90% of IGF-I is bound to IGFBP3 (5). IGFBP1 is inversely regulated by insulin and acutely mediates IGF-I bioavailability (6). Previous work within the Multiethnic Cohort suggests that common genetic variation in *IGF1* is associated with prostate cancer risk but not associated with risk of breast cancer (7, 8). Whether or not common genetic variation in *IGFBPs* is related to prostate and breast cancer risk has not been thoroughly examined. Prior studies have examined a small number of polymorphisms with most focusing on the *IGFBP3* (A-202C) polymorphism as the C allele has been associated with lower circulating levels of IGFBP3 (9-16), aggressive prostate cancer (17), and increased breast cancer risk (9). The majority of these studies were conducted in modest-sized populations (9, 11, 14, 17-19). To systematically follow-up these prior reports, we undertook the first comprehensive evaluation of the genetic diversity of both the *IGFBP1* and *IGFBP3* genes, which are located only 19 kb apart on chromosome 7p13-p12, and conducted two large association studies to test whether inherited differences in these genes influence prostate and breast cancer susceptibility.

Materials and Methods

The Multiethnic Cohort is a large population-based cohort study of >215,000 individuals from Hawaii and California. The

Received 6/10/06; revised 7/13/06; accepted 8/2/06.

Grant support: National Cancer Institute grants CA 63464 and CA 54281, Howard Hughes Medical Institute physician postdoctoral fellowship (M.L. Freedman), and Department of Defense Health Disparity Training-Prostate Scholar Award DAMD # 17-02-1-0246 (M.L. Freedman).

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Note: Supplementary data for this article are available at Cancer Epidemiology Biomakers and Prevention Online (http://cebp.aacrjournals.org/).

Requests for reprints: Matthew Freedman, Program in Medical and Population Genetics, Dana-Farber Cancer Institute, Boston, MA 02115. E-mail: freedman@broad.mit.edu

doi:10.1158/1055-9965.EPI-06-0361

cohort is comprised of predominantly African Americans, Native Hawaiians, Japanese Americans, Latinos, and Whites, who were between the ages of 45 and 75 years when recruited (1993-1996). Further methodologic details of this cohort are provided elsewhere (20).

Our prostate case-control study consists of 2,320 cases and 2,290 controls. The breast case-control study is comprised of 1,615 cases and 1,962 controls, the majority of which are postmenopausal (87% and 82%, respectively). Aggressive prostate cancer was defined as regional and metastatic tumors or localized tumors with Gleason grade >8, and advanced breast cancer was defined as regional and metastatic tumors. *In situ* cases of breast cancer were excluded from the study. Detailed information about these nested case-control studies have been reported previously (7, 21). This study was approved by the Institutional Review Boards at the University of Hawaii and the University of Southern California.

Sequencing. To identify missense single nucleotide polymorphisms (SNP) not in the standard databases, we attempted to sequence the four coding exons of *IGFBP1* and *IGFBP3*, respectively, in 95 aggressive prostate and 95 advanced breast cancer cases (n = 19 per ethnic group). Exon 1 of *IGFBP1* and exon 4 of *IGFBP3* did not meet our sequencing criteria defined as >80% of the samples with phred (base calling) scores >20 for at least 80% of the target bases. Thus, we relied on only publicly available SNP information for these two exons. Further details on sequencing methods are described elsewhere (22).

SNP Selection and Genotyping for Genetic Characterization. We genotyped SNPs spanning 71 kb across the *IGFBP1* (5.3 kb) and *IGBP3* (9.0 kb) loci in a multiethnic panel of 349 controls (Supplementary Fig. S1). We evaluated 19.3 and 18.7 kb upstream of the first exons of *IGFBP1* and *IGFBP3*, respectively, and 46.3 and 43.2 kb downstream of the transcribed region for each gene, respectively. A total of 36 common SNPs (minor allele frequency >5%) was used for genetic characterization with an average density of one SNP every 2.0 kb (Supplementary Table S1). SNP genotyping was done using the Sequenom MASSArray spectrometry platform.

Determination of Linkage Disequilibrium and Tagging SNP Selection. The D' statistic was used to determine the pairwise linkage disequilibrium (LD) between the SNPs. Regions of strong LD (blocks) were defined according to the confidence interval method of Gabriel et al. (23). We aimed to achieve a SNP density such that each block contained at least six high-frequency SNPs (minor allele frequency >10%). Hawaiians, Japanese Americans, Latinos, and Whites shared similar LD patterns with three blocks spanning the *IGFBP1/IGFBP3* locus. We combined these populations to assess the LD structure, and each block had at least six common SNPs. For the African-American population, we genotyped an additional 15 SNPs in regions where there were fewer than six common SNPs in strong LD. These additional SNPs included all dbSNP and Celera available SNPs as of April 2004. After this additional effort, block 3 of this locus contained nine SNPs (minor allele frequency >10%), but blocks 1 and 2 still had fewer than six SNPs. This suggests that among African Americans, we may have incomplete coverage in these regions.

Genotype data for each ethnic group in the multiethnic panel was used to estimate haplotype frequencies within blocks using the expectation-maximization algorithm. The squared correlations (R^2_h) between the true haplotypes (h) and their estimates were estimated as described by Stram et al. (24). For each ethnic group, we selected the minimum set of tagging SNPs (tSNP) within each block for each ethnic group to assure an $R^2_h > 0.7$ for all haplotypes with an estimated frequency > 5%, which we defined as common haplotypes (24).

We sought to include all interblock SNPs as tSNPs to be genotyped in our case-control study. Of the three interblock SNPs between blocks 2 and 3 (SNP 23, 24, and 25), we were unable to design a genotype assay for SNP 25. For the 13 SNPs that were not genotyped in the case-control study, which we refer herein as ''unmeasured SNPs,'' we estimated for each individual the allelic distribution of these unmeasured SNPs by using the 23 tSNPs and genotype data obtained from the multiethnic panel. Within each region of strong LD, we used genotype data from an individual tSNP or a combination of tSNPs to predict each individual's genotype for the unmeasured SNPs by calculating the squared correlations (R^2_s) between each SNP (s) and their estimates obtained from the expectation-maximization algorithm.

Genotyping in Prostate and Breast Cancer Case-Control Studies. The tSNPs were genotyped in patient samples using the 5′ nuclease Taqman allelic discrimination assay. All assays were undertaken by individuals blinded to case-control status. For the prostate samples, the concordance rate for 228 replicate samples was 99.8%, and the average genotyping success was 98.8%. For the breast samples, the concordance rate for 263 replicates was 99.7%, and the average genotyping success was 97.9%. All tSNPs were in Hardy-Weinberg equilibrium ($P > 0.01$ among controls in at least four of the five ethnic groups).

Prostate and Breast Cancer Case-Control Analyses. First, to investigate the hypothesis that genetic susceptibility to cancer risk is associated with single causal variants, we evaluated the relationship between *IGFBP1* and *IGFBP3* genotypes and disease risk. We also considered potential gene-gene interactive effects on prostate cancer risk between *IGFBP1-IGF1* and *IGFBP3-IGF1* by examining the respective multiplicative effects between *IGFBP1* and *IGFBP3* polymorphisms and rs7965399, an *IGF1* polymorphism previously associated with increased prostate cancer risk (P = 0.002) in the Multiethnic Cohort (7). Odds ratios (OR) and 95% confidence intervals (95% CI) were estimated by unconditional logistic regression for the association between genotypes and risk of prostate and breast cancer.

Next, to potentially capture other unmeasured variants that may not be adequately captured by single markers, we evaluated the relationship between common *IGFBP1* and *IGFBP3* haplotypes and cancer risk. Haplotype frequencies among prostate and breast cancer cases and controls were estimated by using tSNP genotype data as described by Stram et al. (24). In brief, for each individual and each haplotype, the haplotype dosage estimate was computed using that individual's genotype data and frequency estimates from the combined case-control data set. First, a global likelihood ratio test was conducted to evaluate the distributions of haplotypes between cases and controls. ORs and 95% CIs were then estimated by unconditional logistic regression for the association between cancer risk and each common haplotype within blocks using 0 copy for each haplotype as the reference.

ORs were adjusted for age and also for ethnicity in any analysis that combined the five ethnic groups. We tested for heterogeneity of haplotype effects across ethnic groups by including an interaction term between haplotype and ethnicity in a multivariate model. All of the following results were similar when adjustment was made for family history of cancer, and breast cancer results were similar when adjusted for established breast cancer risk factors (25).

We conducted permutation testing to guide interpretation of nominally significant SNP associations. Case-control status within strata of age and ethnicity was randomly permuted 10,000 times for the 36 SNPs, and the minimum nominal P at each permutation was selected to generate a null distribution. We calculated that a P of 0.001 marked the 5th percentile of the generated distribution (i.e., an α level <0.05), which we used as the statistical threshold to declare significance.

Table 1. Odds Ratios and 95% CIs of prostate and breast cancer for *IGFBP3* (A-202C)

IGFBP3 (A-202C)	Prostate cancer analysis			Breast cancer analysis		
	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)	Cases, *n* (%)	Controls, *n* (%)	OR (95% CI)
All*						
AA	687 (30.1)	720 (31.9)	1.00	502 (32.1)	598 (31.2)	1.00
AC	1002 (44.0)	954 (42.3)	1.09 (0.95-1.26)	673 (43.0)	891 (46.4)	0.94 (0.79-1.10)
CC	590 (25.9)	581 (25.8)	1.06 (0.90-1.26)	389 (24.9)	431 (22.5)	1.15 (0.94-1.41)
African Americans†						
AA	217 (32.6)	224 (34.9)	1.00	97 (29.9)	132 (31.6)	1.00
AC	308 (46.3)	298 (46.4)	1.07 (0.84-1.37)	115 (47.8)	209 (50.0)	1.01 (0.72-1.41)
CC	141 (21.2)	120 (18.7)	1.22 (0.90-1.66)	72 (22.2)	77 (18.4)	1.28 (0.84-1.93)
Hawaiians†						
AA	22 (31.4)	24 (35.8)	1.00	29 (28.2)	97 (34.3)	1.00
AC	36 (52.2)	33 (49.3)	1.14 (0.53-2.42)	51 (49.5)	121 (42.8)	1.36 (0.80-2.32)
CC	12 (17.1)	10 (14.9)	1.23 (0.44-3.46)	23 (22.3)	65 (23.0)	1.14 (0.61-2.16)
Japanese Americans†						
AA	264 (58.0)	282 (60.5)	1.00	254 (60.9)	234 (56.9)	1.00
AC	161 (35.4)	161 (34.6)	1.05 (0.80-1.39)	138 (33.1)	158 (38.4)	0.81 (0.61-1.08)
CC	30 (6.6)	23 (4.9)	1.37 (0.77-2.42)	25 (6.0)	19 (43.2)	1.19 (0.64-2.22)
Latinos†						
AA	81 (12.7)	95 (14.9)	1.00	45 (13.6)	53 (14.0)	1.00
AC	277 (43.5)	257 (40.4)	1.26 (0.89-1.77)	147 (44.6)	172 (45.3)	1.02 (0.65-1.61)
CC	279 (43.8)	284 (44.7)	1.15 (0.82-1.62)	138 (41.8)	155 (40.8)	1.06 (0.67-1.69)
Whites†						
AA	103 (22.8)	95 (21.4)	1.00	77 (19.7)	82 (19.2)	1.00
AC	220 (48.8)	205 (46.2)	0.98 (0.70-1.38)	182 (46.7)	231 (54.0)	0.84 (0.58-1.21)
CC	128 (28.4)	144 (32.4)	0.81 (0.56-1.17)	131 (33.6)	115 (26.9)	1.25 (0.84-1.88)

NOTE: *IGFBP3* (A-202C) (rs2854744) A/C alleles are of the reverse strand.
*ORs are estimated by unconditional logistic regression adjusted for age and ethnicity.
† ORs are estimated by unconditional logistic regression adjusted for age.

Results

Genetic Characterization of the *IGFBP1* and *IGFBP3* Locus. No common missense polymorphisms were discovered by sequencing the exons of *IGFBP1* and *IGFBP3* that were not already present in dbSNP. We identified three regions of strong LD among Hawaiians, Japanese Americans, Latinos, and Whites: block 1 (SNPs 2-9, size = 19 kb) spanned the upstream region of *IGFBP1*; block 2 (SNPs 10-22, size = 21 kb) included the region of intron 1 to 3′ downstream of *IGFBP1*;

Table 2. Associations between common haplotypes in blocks 1 to 3 of *IGFBP1* and I*GFBP3* and prostate cancer risk

	Haplotype percentages in cases/controls (%)					OR (95% CI)	
	African Americans (*n* = 683/650†)	Native Hawaiians (*n* = 71/68†)	Japanese Americans (*n* = 462/471†)	Latinos (*n* = 647/649†)	Whites (*n* = 457/452†)	All groups (0 vs 1 copy)*	All groups (0 vs 2 copies)*
Block 1							
1A GGCGA	20/20	16/17	15/17	16/17	20/18	0.99 (0.87-1.13)	0.92 (0.67-1.27)
1B AGCGG	11/10	19/20	14/16	22/26	25/27	0.89 (0.78-1.01)	0.85 (0.64-1.15)
1C AGGGG	28/27	18/25	30/25	25/22	24/24	1.11 (0.97-1.25)	1.18 (0.93-1.48)
1D AACGA	7/9	30/21	24/24	21/20	17/19	0.97 (0.85-1.11)	1.02 (0.74-1.41)
1E GGCAG	15/15	11/12	6/7	4/4	3/2	0.99 (0.83-1.19)	0.80 (0.40-1.59)
1F GGCGG	4/4	4/2	9/7	10/9	9/9	1.23 (1.03-1.47)‡	0.95 (0.44-2.03)
1G AGCGA	8/8	0/0	0/0	0/0	0/0	0.99 (0.72-1.35)	4.97 (0.57-43.23)
Block 2							
2A ACACATTTC	30/28	30/38	32/35	26/23	27/25	1.09 (0.96-1.23)	1.23 (0.99-1.53)
2B ACACACTTC	24/23	14/18	22/23	32/34	33/33	0.95 (0.84-1.09)	0.90 (0.72-1.12)
2C GCACGCTTC	6/6	36/23	26/27	19/18	15/16	1.03 (0.90-1.19)	1.07 (0.76-1.50)
2D GCGCGCCGT	5/3	9/12	12/13	7/9	14/13	1.07 (0.91-1.25)	0.48 (0.25-0.94)‡
2E GTACGCTGT	0/0	0/0	0/0	0/0	0/0	—	—
2F GCATGCTTT	14/15	0/0	0/0	0/0	0/0	0.93 (0.73-1.19)	0.87 (0.42-1.78)
2G GCGCGCCGC	0/0	0/0	0/0	0/0	0/0	—	—
2H GCATGCCGT	0/0	0/0	0/0	7/7	0/0	0.99 (0.78-1.27)	0.26 (0.05-1.28)
Block 3							
3A GGACTGT	33/32	54/57	75/76	30/31	40/38	1.03 (0.90-1.18)	1.00 (0.84-1.20)
3B AGGGGTT	7/4	13/10	11/10	41/41	19/24	1.07 (0.92-1.23)	0.94 (0.73-1.21)
3C AGGGGGG	13/14	13/17	1/1	16/16	20/21	0.91 (0.78-1.05)	0.98 (0.66-1.46)
3D GGAGTGT	20/22	2/3	0/0	4/4	6/4	0.93 (0.77-1.12)	0.99 (0.59-1.64)
3E GGAGGGT	8/7	2/2	0/0	6/6	11/10	1.12 (0.93-1.35)	1.96 (0.83-4.62)
3F AAGGGTT	5/4	14/11	12/11	1/1	1/1	1.08 (0.86-1.35)	1.47 (0.59-3.70)
3G AGGGGGT	7/8	0/0	0/0	1/1	0/0	1.02 (0.76-1.38)	1.28 (0.19-8.63)

NOTE: ORs are estimated by unconditional logistic regression adjusted for age and ethnicity.
*Reference groups for each comparison are noncarriers of each haplotype.
† *n* = cases/controls.
‡ $P < 0.05$.

block 3 (SNPs 26-36, size = 25 kb) spanned intron 3 of *IGFBP3* to 5′ upstream (Supplementary Fig. S1). The distances between adjacent blocks were <5 kb.

The tSNPs in each block were able to capture all common haplotypes with an $R^2_h > 0.9$. Within each block, the common haplotypes for each ethnic group accounted for 76% to 100% of the chromosomes in the multiethnic panel population. The 23 tSNPs predicted the 13 unmeasured SNPs in the case-control study with an average R^2_s of 0.88.

Prostate Cancer Association Study. We observed no nominally significant associations ($P > 0.05$) between any of the 36 *IGFBP1* and *IGFBP3* SNPs (23 tSNPs and 13 unmeasured SNPs) and prostate cancer risk (data not shown). Table 1 presents the association between the previously associated *IGFBP3* A-202C polymorphism (rs2854744) and prostate cancer risk. We found no evidence that the prostate cancer effects of the previously associated *IGF1* polymorphism, rs7965399 (7), were modified by either *IGFBP1* or *IGFBP3* variants (data not shown).

Table 2 presents the associations between prostate cancer risk and common *IGFBP1* and *IGFBP3* haplotypes. A marginally statistically significant haplotype effect in block 1 was observed for prostate cancer risk (global test, $P = 0.05$). Heterozygous carriers of haplotype 1F had a nominally statistically significant increased risk of prostate cancer (OR, 1.23; 95% CI, 1.03-1.47; $P = 0.03$) compared with noncarriers. When stratified by disease aggressiveness, this association remained nominally significant for nonaggressive disease (OR, 1.25; 95% CI, 1.02-1.53; $P = 0.03$) and aggressive prostate cancer (OR, 1.31; 95% CI, 1.02-1.67; $P = 0.03$). Racial/ethnic–stratified analysis revealed an overall consistent positive pattern across all five groups (power was decreased, however, due to the smaller numbers; data not shown). No significant global haplotype effects were observed in block 2 ($P = 0.38$) or block 3 ($P = 0.55$). No associations were observed with individual haplotypes within block 2 or 3. We tested for interactive effects between haplotype and ethnic group and found no significant interactions ($P = 0.05$-0.97).

Breast Cancer Association Study. No nominally significant associations ($P > 0.05$) were observed between any of the 36 *IGFBP1* and *IGFBP3* SNPs (23 tSNPs and 13 unmeasured SNPs) and breast cancer risk (data not shown). The association between the *IGFBP3* A-202C polymorphism and breast cancer risk is presented in Table 1. Table 3 presents the associations between breast cancer risk and common *IGFBP1* and *IGFBP3* haplotypes. There were no significant global haplotype effects in any of the three blocks: block 1 ($P = 0.63$), block 2 ($P = 0.85$), and block 3 ($P = 0.65$). Nominally statistically significant associations were observed with haplotypes 1E and 3B. Rare homozygous carriers of haplotype 1E compared with noncarriers displayed a nominally significant association with increased breast cancer risk (OR, 2.17; 95% CI, 1.10-4.29; $P = 0.03$), and a nonsignificant positive association was observed with advanced disease (OR, 1.69; 95% CI, 0.53-5.35; $P = 0.37$). Heterozygous carriers of haplotype 3B had an estimated 1.23-fold increased risk of breast cancer compared with noncarriers (95% CI, 1.05-1.44; $P = 0.01$), whereas among advanced breast cancer cases, no association was seen (OR, 0.97; 95% CI, 0.75-1.26; $P = 0.84$). We found no significant interactions between haplotype and ethnicity effects ($P = 0.09$-0.92).

Discussion

In the present study, we characterized the genetic variation in *IGFBP1* and *IGFBP3* and tested whether inherited differences in these genes influence prostate and breast cancer susceptibility in two large multiethnic case-control studies. We

Table 3. Associations between common haplotypes in blocks 1 to 3 of *IGFBP1* and *IGFBP3* and breast cancer risk

	Haplotype percentages in cases/controls (%)					OR (95% CI)	
	African Americans (n = 345/426 †)	Native Hawaiians (n = 109/209 †)	Japanese Americans (n = 425/420 †)	Latinos (n = 335/386 †)	Whites (n = 401/440 †)	All groups (0 vs 1 copy) *	All groups (0 vs 2 copies) *
Block 1							
1A GGCGA	16/19	20/15	16/19	20/17	18/19	1.03 (0.88-1.20)	0.83 (0.58-1.20)
1B AGCGG	11/11	14/17	15/13	25/25	25/27	0.91 (0.78-1.06)	1.22 (0.86-1.75)
1C AGGGG	29/27	18/22	27/29	22/25	23/22	0.88 (0.76-1.02)	1.03 (0.78-1.37)
1D AACGA	8/9	25/26	25/24	17/19	21/20	1.00 (0.86-1.16)	0.89 (0.63-1.26)
1E GGCAG	15/15	15/13	6/5	4/3	3/3	1.06 (0.86-1.30)	2.17 (1.10-4.29) ‡
1F GGCGG	4/3	6/4	7/8	8/9	9/8	1.03 (0.83-1.27)	4.32 (0.87-21.35)
1G AGCGA	7/8	1/1	—	1/1	—	0.94 (0.62-1.43)	0.49 (0.06-4.25)
Block 2							
2A ACACATTTC	31/29	35/34	33/34	26/24	26/26	0.95 (0.83-1.10)	1.19 (0.93-1.52)
2B ACACACTTC	23/23	14/16	23/20	33/37	32/33	0.97 (0.84-1.13)	0.98 (0.75-1.27)
2C GCACGCTTC	7/7	31/32	28/26	15/17	19/17	1.01 (0.87-1.18)	1.03 (0.73-1.47)
2D GCGCGCCGT	3/3	8/8	13/13	9/7	13/13	1.08 (0.90-1.30)	0.74 (0.37-1.51)
2E GTACGCTGT	5/7	0/0	0/0	0/0	0/0	0.78 (0.50-1.21)	—
2F GCATGCTTT	12/12	0/0	0/0	1/1	0/0	1.04 (0.75-1.45)	0.89 (0.28-2.83)
2G GCGCGCCGC	0/0	4/5	1/2	0/0	0/0	0.71 (0.41-1.22)	—
2H GCATGCCGT	1/1	3/2	0/0	8/7	4/4	1.14 (0.84-1.54)	0.57 (0.14-2.33)
2I GCACGCCGT	5/5	0/0	0/0	0/0	0/0	0.80 (0.49-1.31)	—
Block 3							
3A GGACTGT	33/31	49/52	76/75	31/32	36/40	0.93 (0.79-1.09)	0.93 (0.76-1.14)
3B AGGGGTT	8/6	16/13	11/11	40/39	22/20	1.23 (1.05-1.44) ‡	1.01 (0.72-1.41)
3C AGGGGGG	12/12	10/14	1/1	17/19	21/20	0.89 (0.75-1.06)	1.31 (0.81-2.11)
3D GGAGTGT	18/21	2/2	0/0	4/4	5/4	0.85 (0.67-1.09)	0.98 (0.48-2.01)
3E GGAGGGT	8/8	4/5	0/0	5/4	13/12	0.98 (0.78-1.22)	1.82 (0.74-4.45)
3F AAGGGTT	3/4	17/11	10/11	1/1	1/1	0.97 (0.76-1.25)	1.94 (0.79-4.77)
3G AGGGGGT	8/7	0/0	0/0	0/0	0/0	1.27 (0.84-1.90)	0.37 (0.04-3.03)

NOTE: ORs are estimated by unconditional logistic regression adjusted for age and ethnicity.
*Reference groups for each comparison are noncarriers of each haplotype.
† n = cases/controls.
‡ $P < 0.05$.

identified three regions of strong LD and chose 23 tSNPs that captured the common genetic variation in *IGFBP1* and *IGFBP3*. No statistically significant associations between individual SNPs with either prostate or breast cancer risk were observed. Weak haplotype associations were seen with prostate and breast cancer risk. However, given the magnitude of the *P*s (~0.05) observed, the positive signals observed in this study are likely to be false positives and are not unexpected given the number of hypotheses (i.e., alleles) tested. We determined that a corrected α of 0.001 was needed to declare statistical significance. Our study had 80% power for both the prostate and breast studies to detect a minimum OR of 1.40 for a 10% haplotype at an α level of 0.001 (two-sided hypothesis test), assuming a codominant model (Quanto software, Los Angeles, CA; ref. 26). Our data suggest that common genetic variation at the *IGFBP1* and *IGFBP3* loci does not substantially influence prostate and breast cancer risk.

The *IGFBP3* polymorphism (A-202C) has been most often studied as lower circulating levels of IGFBP-3 have been associated with the C allele (9-14). Four studies examined the association between *IGFBP3* (A-202C) and prostate cancer risk, with one study finding a nonsignificant increased risk associated with the C allele among African Americans (18) and the remaining studies observing no association (14, 17, 19). In a study of 307 Japanese prostate cancer cases, Wang et al. observed that the C allele of *IGFBP3* (A-202C) was correlated with advanced disease compared with localized disease (17). We found no association (P = 0.74) between this polymorphism and risk of either aggressive prostate or nonaggressive prostate, and similar findings were observed in racial/ethnic–stratified analysis. Of the four studies of breast cancer (9, 11, 15, 16), one study reported a positive association with the C allele of *IGFBP3* (A-202C; ref. 9), whereas the other study found no association (11). Results from our large studies of prostate and breast confirm and extend previous studies that showed no association between the *IGFBP3* (A-202C) polymorphism and cancer risk (14-19). Our studies had 98% and 91% power, with 2,320 prostate cancer cases and 1,615 breast cancer cases, respectively, to detect a minimum OR of 1.20 for this polymorphism (codominant model, α = 0.05, two-sided hypothesis test).

In summary, our data do not support the involvement of common genetic variation in *IGFBP1* and *IGFBP3* with either prostate or breast cancer risk. As circulating levels of IGFBP-3 may be associated with cancer risk, it is possible that genetic regions outside of *IGFBP1/IGFBP3* as well as interactive effects between gene and environment may influence IGFBP-3 levels.

Acknowledgments

We thank the participants of the Multiethnic Cohort who have contributed to a better understanding of the genetic contributions to cancer susceptibility; Noel Burtt, Loreall Pooler, Stephanie Riley, and David Wong for their laboratory assistance; and Kris Monroe and Hank Huang for their technical support.

References

1. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363:1346–53.
2. Helle SI. The insulin-like growth factor system in advanced breast cancer. Best Pract Res Clin Endocrinol Metab 2004;18:67–79.
3. Pollak M. Insulin-like growth factors and prostate cancer. Epidemiol Rev 2001;23:59–66.
4. Latif Z, McMillan DC, Wallace AM, et al. The relationship of circulating insulin-like growth factor 1, its binding protein-3, prostate-specific antigen and C-reactive protein with disease stage in prostate cancer. BJU Int 2002;89:396–9.
5. Baxter RC. Insulin-like growth factor binding proteins in the human circulation: a review. Horm Res 1994;42:140–4.
6. Lee PD, Giudice LC, Conover CA, Powell DR. Insulin-like growth factor binding protein-1: recent findings and new directions. Proc Soc Exp Biol Med 1997;216:319–57.
7. Cheng I, Stram DO, Penney KL, et al. Common genetic variation in IGF1 and prostate cancer risk in the Multiethnic Cohort. J Natl Cancer Inst 2006;98:123–34.
8. Setiawan VW, Cheng I, Stram DO, et al. Igf-I genetic variation and breast cancer: the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 2006;15:172–4.
9. Ren Z, Cai Q, Shu XO, et al. Genetic polymorphisms in the IGFBP3 gene: association with breast cancer risk and blood IGFBP-3 protein levels among Chinese women. Cancer Epidemiol Biomarkers Prev 2004;13:1290–5.
10. Deal C, Ma J, Wilkin F, et al. Novel promoter polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. J Clin Endocrinol Metab 2001;86:1274–80.
11. Schernhammer ES, Hankinson SE, Hunter DJ, Blouin MJ, Pollak MN. Polymorphic variation at the −202 locus in IGFBP3: influence on serum levels of insulin-like growth factors, interaction with plasma retinol and vitamin D and breast cancer risk. Int J Cancer 2003;107:60–4.
12. Le Marchand L, Kolonel LN, Henderson BE, Wilkens LR. Association of an exon 1 polymorphism in the IGFBP3 gene with circulating IGFBP-3 levels and colorectal cancer risk: the Multiethnic Cohort Study. Cancer Epidemiol Biomarkers Prev 2005;14:1319–21.
13. Jernstrom H, Deal C, Wilkin F, et al. Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor-binding protein-3 in healthy premenopausal women. Cancer Epidemiol Biomarkers Prev 2001;10:377–84.
14. Nam RK, Zhang WW, Trachtenberg J, et al. Comprehensive assessment of candidate genes and serological markers for the detection of prostate cancer. Cancer Epidemiol Biomarkers Prev 2003;12:1429–37.
15. Al-Zahrani A, Sandhu MS, Luben RN, et al. IGF1 and IGFBP3 tagging polymorphisms are associated with circulating levels of IGF1, IGFBP3 and risk of breast cancer. Hum Mol Genet 2006;15:1–10.
16. Canzian F, McKay JD, Cleveland RJ, et al. Polymorphisms of genes coding for insulin-like growth factor 1 and its major binding proteins, circulating levels of IGF-I and IGFBP-3 and breast cancer risk: results from the EPIC study. Br J Cancer 2006;94:299–307.
17. Wang L, Habuchi T, Tsuchiya N, et al. Insulin-like growth factor-binding protein-3 gene −202 A/C polymorphism is correlated with advanced disease status in prostate cancer. Cancer Res 2003;63:4407–11.
18. Schildkraut JM, Demark-Wahnefried W, Wenham RM, et al. IGF1 (CA)19 repeat and IGFBP3 -202 A/C genotypes and the risk of prostate cancer in Black and White men. Cancer Epidemiol Biomarkers Prev 2005;14:403–8.
19. Li L, Cicek MS, Casey G, Witte JS. No association between genetic polymorphisms in IGF-I and IGFBP-3 and prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13:497–8.
20. Kolonel LN, Henderson BE, Hankin JH, et al. A multiethnic cohort in Hawaii and Los Angeles: baseline characteristics. Am J Epidemiol 2000;151:346–57.
21. Haiman CA, Stram DO, Pike MC, et al. A comprehensive haplotype analysis of CYP19 and breast cancer risk: the Multiethnic Cohort. Hum Mol Genet 2003;12:2679–92.
22. Freedman ML, Pearce CL, Penney KL, et al. Systematic evaluation of genetic variation at the androgen receptor locus and risk of prostate cancer in a multiethnic cohort study. Am J Hum Genet 2005;76:82–90.
23. Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. Science 2002;296:2225–9.
24. Stram DO, Haiman CA, Hirschhorn JN, et al. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. Hum Hered 2003;55:27–36.
25. Pike MC, Kolonel LN, Henderson BE, et al. Breast cancer in a multiethnic cohort in Hawaii and Los Angeles: risk factor-adjusted incidence in Japanese equals and in Hawaiians exceeds that in whites. Cancer Epidemiol Biomarkers Prev 2002;11:795–800.
26. Gauderman WJ. Sample size requirements for association studies of gene-gene interaction. Am J Epidemiol 2002;155:478–84.

Haplotype-Based Association Studies of *IGFBP1* and *IGFBP3* with Prostate and Breast Cancer Risk: The Multiethnic Cohort

Iona Cheng, Kathryn L. Penney, Daniel O. Stram, et al.

Cancer Epidemiol Biomarkers Prev 2006;15:1993-1997.