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Eighteen Insulin-like Growth Factor (IGF) pathway genes, circulating levels of IGF-1 and its binding protein (IGFBP-3), and risk of prostate and breast cancer

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Abstract

Background—Circulating levels of insulin-like growth factor I (IGF-1) and its main binding protein, IGF binding protein 3 (IGFBP-3), have been associated with risk of several types of cancer. Heritable factors explain up to 60% of the variation in IGF-1 and IGFBP-3 in studies of adult twins.

Methods—We systematically examined common genetic variation in 18 genes in the IGF signaling pathway for associations with circulating levels of IGF-1 and IGFBP-3. A total of 302 single nucleotide polymorphisms (SNPs) were genotyped in over 5500 Caucasian men and 5500 Caucasian women from the Breast and Prostate Cancer Cohort Consortium (BPC3).

Results—After adjusting for multiple testing, SNPs in the *IGF1* and *SSTR5* genes were significantly associated with circulating IGF-1 ($p<2.1\times10^{-4}$); SNPs in the *IGFBP3* and *IGFALS* genes were significantly associated with circulating IGFBP-3. Multi-SNP models explained R^2=0.62% of the variation in circulating IGF-1 and 3.9% of the variation in circulating IGFBP-3. We saw no significant association between these multi-SNP predictors of circulating IGF-1 or IGFBP-3 and risk of prostate or breast cancers.

Conclusion—Common genetic variation in the *IGF1* and *SSTR5* genes appears to influence circulating IGF-1 levels, and variation in *IGFBP3* and *IGFALS* appears to influence circulating IGFBP-3. However, these variants explain only a small percentage of the variation in circulating IGF-1 and IGFBP-3 in Caucasian men and women.

Impact—Further studies are needed to explore contributions from other genetic factors such as rare variants in these genes and variation outside of these genes.

Keywords

insulin-like growth factors; genetic association; breast cancer; prostate cancer

INTRODUCTION

The insulin-like growth factor (IGF) signaling pathway plays an important role in regulating cellular proliferation and apoptosis (1). IGF-1 and its major binding protein, IGFBP3, are two

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of the key molecules in this pathway. Epidemiological studies suggest a positive association between levels of circulating IGF-1 and risk of prostate breast cancers(2–4). Associations between circulating IGFBP-3 and prostate and breast cancers are inconsistent (2,4–5).

The IGF signaling pathway is a complex regulatory system (Fig 1). The main components include IGF-1, IGF-2, the two IGF receptors (IGF-1R and IGF-2R), the six binding proteins [IGFBP-1 through 6], the acid labile subunit (ALS), and the upstream and downstream regulatory factors. The expression of both IGF-1 and IGFBP-3 are upregulated by growth hormone (GH), whose expression is under the regulation of the hypothalamic hormones somatostatin (SST, an inhibitor) and growth hormone-releasing hormone (GHRH, a stimulator). The pituitary-specific transcription factor 1 (POU1F1) is crucial for the synthesis of GH in the pituitary gland. Growth hormone receptor (GHR), growth hormone-releasing hormone receptor (GHRHR), and five somatostatin receptors (SSTR-1 through 5) bind their respective ligands and regulate their function (6).

In addition to age, gender, smoking, and nutrition, genetic factors may also influence circulating levels of both IGF-1 and IGFBP-3. Twin studies have shown up to 60% of IGF-1 and IGFBP-3 may be explained by genetic factors in adults (7–8).

Genetic variation in these IGF signaling pathway genes may influence the concentration of IGF-1 and IGFBP-3 in circulation. Epidemiologic studies that have correlated IGF-1 and IGFBP-3 levels with variants in *IGF1*(9–12), *IGFBP1* (10), *IGFBP3* (9–11), *IGFALS* (10), and 10 GH-related genes (*GHRH*, *GHRHR*, *SST*(13), *SSTR1-SSTR5*(13–14), *POU1F1*, and *GH*)(15) have generally assessed only a few single nucleotide polymorphisms (SNPs) within either one or several genes in this pathway. Most of these studies had insufficient power to detect subtle associations, and SNP panels differed across studies. For many frequently studied SNPs the results are inconsistent (11).

We comprehensively examined the association between common variants in 18 IGF signaling pathway genes and circulating levels of IGF-1 and IGFBP-3 using data collected by the Breast and Prostate Cancer Cohort Consortium (BPC3), a collaboration of nine large prospective studies. The BPC3 genotyped 302 tagging SNPs in these genes and measured circulating levels of IGF-1 and IGFBP-3 in six male and four female nested case-control studies. Our sample size (over 5500 Caucasian women and 5500 Caucasian men) is among the largest to date to examine these associations. The BPC3 previously reported associations between common genetic variation in the *IGF1*, *IGFBP1*, and *IGFBP3* genes and circulating levels of IGF-1 and IGFBP-3 in men (16)and women (17) separately. We extend this analysis to include 15 other genes in the IGF signaling pathway: *GHR*, *GHRH*, *GHRHR*, *IGFALS*, *IGFBP2*, *IGFBP4-6*, *POU1F1*, *SST*, and *SSTR1-5*. (Fig 1) In separate publications we assess whether the tagging SNPs in these genes are individually associated with risk of prostate or breast cancer (16–18); here we test whether markers with strong statistical evidence for association with circulating IGF-1 and IGFBP-3 are collectively associated with risk of prostate or breast cancer.

MATERIALS AND METHODS

Study Population

As described previously (19), the BPC3 pooled data from nine well-established cohorts from the United States and Europe to study the association between variation in hormone-related genes and risk of breast and prostate cancers. Each member cohort provided data on prostate/breast cancer cases and their matched controls; seven member cohorts contributed prospectively collected blood samples for hormone assay. In this analysis we used subjects with both successful genotyping data and blood IGF-1 (or IGFBP-3) assay to examine the association of IGF pathway genes with circulating IGF-1 (or IGFBP-3) level. We restricted

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the analyses to Caucasians, who comprised the majority of participants in all except the Multiethnic Cohort (MEC) (20). Prediagnostic measures of circulating IGF-1 were available from 5583 Caucasian men (2664 prostate cancer patients and 2919 controls) from 6 cohorts and 5533 Caucasian women (2080 breast cancer patients and 3453 controls) from 4 cohorts; data on circulating IGFBP-3 were available from 5565 Caucasian men (2661 cases and 2904 controls) and 5420 Caucasian women (2044 cases and 3376 controls). The 6 male cohorts included the Physicians' Health Study (PHS) (21), the Health Professionals Follow-Up Study (HPFS) (22), the European Prospective Investigation into Cancer and Nutrition (EPIC) Study (23), the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial (24), the Alpha-Tocopherol, Beta-Carotene Cancer Prevention (ATBC) Study (25), and the MEC Study. The 4 female cohorts were the Nurses' Health Study (NHS) (26), the EPIC, the PLCO, and the MEC.

Cases were initially identified in each cohort by self-report or cancer registries and subsequently confirmed with medical records, including pathological reports. Controls were selected matching on a number of potential confounding factors: age and ethnicity, and in some cohorts, additional criteria, such as region of recruitment in EPIC (16–17). Questionnaire data were collected prospectively (i.e. prior to cancer diagnosis). Informed consent was obtained from each individual, and each study was approved by the Institutional Review Board at the respective institutions.

IGF-1 and IGFBP-3 Circulating Levels

Circulating IGF-1 and IGFBP-3 levels were measured by Enzyme-Linked Immunosorbent Assays (Diagnostic System Laboratories, Webster, TX). All of these measurements were made using blood samples collected before diagnosis with cancer, except in the MEC, where the one breast cancer case was prevalent at blood draw. To account for possible reverse causality due to latent cancer, we repeated all association analyses restricting to controls and cases diagnosed more than two years after blood draw (9,164 men and women combined). All the female subjects were non-users of menopausal hormone therapy (and non-users of oral contraceptives in EPIC) at the time of blood draw. Samples from ATBC, HPFS, PHS, NHS and PLCO-women were measured in the Michael Pollak's laboratory (McGill University), and samples from the remaining studies (EPIC, MEC, and PLCO-men) were measured in the laboratory of the Hormones and Cancer Team at the International Agency for Research on Cancer (IARC). The detailed measurements are described elsewhere (21,27–32).

SNP Discovery and htSNP Selection

SNP discovery and selection of tagging SNPs were based on two approaches, depending on the maturity of the HapMap database at the time tagging SNPs were chosen. For three genes (*IGF1*, *IGFBP1*, and *IGFBP3*), studied before the completion of the HapMap Project, novel SNPs were identified by resequencing coding and evolutionarily conserved regions in a discovery panel of 190 advanced prostate and breast cancer cases from the MEC samples (19 for each cancer and each of five ethnic groups: African American, Latino, Japanese, Native Hawaiian, and Caucasian). A dense set of SNPs in each gene in a multiethnic, cancer-free panel (including 70 MEC Caucasians) was used to determine the linkage disequilibrium (LD) patterns for further use. This set included SNPs with minor allele frequency (MAF) >0.05 from public databases spanning the region from 20 kb upstream of the start of transcription to 10 kb downstream of the end of transcription; it also included novel SNPs detected by resequencing with MAF greater than 5% in any of the five ethnic groups or greater than 1% overall. Using this dense genotyping data, haplotype blocks were determined by a modified version of the Gabriel et al. algorithm (33) in the Haploview program (34). Haplotype-tagging SNPs (htSNPs) were then chosen within these blocks with $R_h^2>=0.7$, calculated by the partition-ligation expectation maximization (PLEM) algorithm in the tagSNPs program (35). A different

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approach was used to characterize variants in other IGF pathway genes. For these, pairwise tagging SNPs were selected using the HapMap Phase 2 CEU (European ancestry) panel and the algorithm implemented in Tagger (36–38). Tag SNPs were chosen so that all SNPs with MAF>0.05 were tagged with r^2>0.8. Detailed information on the resequencing results in the discovery panel and on the dense panel of SNPs genotyped in the reference panel can be found at the BPC3 website (39).

Genotyping

Two methods were used to genotype these SNPs: the fluorogenic 5' endonuclease assay (TaqMan), using reagents and hardware from Applied Biosystems (Foster City, CA, USA) for the *IGF1*, *IGFBP1*, and *IGFBP3* genes; the Illumina GoldenGate assay (Illumina, San Diego, CA, USA) was used for the others. Genotyping was performed in eight laboratories (University of Southern California, Los Angeles, CA, USA; University of Hawaii, Honolulu, HI, USA; Harvard School of Public Health, Boston, MA, USA; Core Genotyping Facility, National Cancer Institute, Bethesda, MD, USA; Cambridge University, Cambridge, UK; International Agency for Research on Cancer, Lyon, France; Imperial College, London, UK and German Cancer Research Center, Heidelberg, German). Inter-laboratory reproducibility was assessed with 30 SNPs on 94 samples from the Coriell Biorepository (Camden, NJ); the concordance rates were greater than 99% for Taqman assay, and 99.5% for the Illumina OPA assay. The internal quality of each genotyping center was assessed by typing 5–10% blinded samples in duplicate or greater, and the concordance ranged from 97.2 to 99.9%.

Data cleaning and imputation

Samples in which more than 25% of attempted SNPs failed were excluded. Less than 3% of samples were excluded due to low completion rate; the study-specific exclusion rates were less than 5% for all cohorts, except the WHS, which had a 9.5% exclusion rate. Also excluded from each cohort were SNPs with high failure rate (>=25%), departures from Hardy-Weinberg Equilibrium among controls ($p < 10^{-5}$), and those with MAF < 0.01. SNPs that failed in more than three cohorts or differed greatly in European-ancestry allele frequencies across cohorts (fixation index F_{st}>0.02) were also excluded. The percentage of attempted SNPs that passed the filters described above ranged between 86.0% and 93.5%, calculated by cohort, and most failures were due to low call rates (<75% in a given cohort). Fewer than 1% of the SNPs were excluded from further analysis because of departure from Hardy-Weinberg Equilibrium ($p<10^{-5}$). Allele frequencies were similar across the cohorts; only two SNPs showed F_{st} values higher than 2%. See supplementary tables in Canzian et al. (18)for more detail.

Failed SNPs were imputed by study and gender using observed genotypes from the BPC3 subjects and phased HapMap CEU samples (release #22) with the software MACH (40–41). Imputed SNPs were excluded from analysis if the estimated correlation between the imputed and true underlying genotypes was less than 30%. The average estimated correlation with the underlying genotypes among SNPs retained for analysis was 0.72; only five SNPs had an estimated correlation less than 0.5. In the combined sample of men and women, we restricted analyses to SNPs that were available for both genders (i.e., SNPs for which genotyping had been attempted and were either genotyped or imputed successfully in both men and women).

Statistical Analysis

Before performing analyses we deleted the batch-specific outliers for circulating IGF-1 and IGFBP-3 levels among both males and females. The outliers were identified based on the generalized extreme studentized deviate many-outlier detection approach, setting alpha to 0.05 (42). This led to the exclusion of 25 IGF-1 samples and 47 IGFBP-3 samples with levels between 1.80 and 6.26 (IGF-1) and 2.35 and 11.16 (IGFBP-3) standard deviations from their respective batch means. The circulating IGF-1 and IGFBP-3 levels were log-transformed to

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provide approximate normal distributions. We used linear regression to examine the association between each single SNP (coded as 0, 1, or 2 for the number of minor alleles present) and the log-transformed IGF-1 or IGFBP-3 level, adjusting for age at blood draw, batch, and case/control status (prostate cancer for males and breast cancer for females). All the analyses were restricted to Caucasians. We performed the analyses within male cohorts and female cohorts separately and then combined them using fixed-effect meta-analyses (43). Percent change of IGF-1 and IGFBP-3 per copy of minor allele for each SNP was calculated as $(e^{\beta} - 1) \times 100\%$, where the β is the per-allele change in mean log biomarker level. Heterogeneity in SNP-hormone associations between men and women was assessed using Cochrane's *Q* statistic (44). Multiple comparison adjustments were performed at the gene level by multiplying nominal p values by the effective number of independent SNPs within each gene (45); because this procedure accounts for correlations among the tested SNPs, it is slightly less conservative than a Bonferroni correction, which assumes the SNPs are independent. We also adjusted for multiple comparisons at the pathway level; the adjusted significance criteria ($p<2.1\times10^{-4}$) was calculated by dividing 0.05 by the sum of the effective number of independent SNPs across all genes (M_{eff}=238).

Genomic control inflation factors λ_{GC} were calculated to assess systematic bias due to residual population stratification or departures from analytic assumptions (e.g., homoscedasticity). (The factor λ_{GC} is calculated as the median of the observed chi-squared test statistics for marker-trait association divided by its theoretical median under the null, which is 0.45 for a one degree of freedom test.) We also used the genomic control inflation factor to correct for potential systematic biases by calculating corrected chi-square statistics Z^2/λ_{GC}, where *Z* is the Wald statistic from the linear regression of IGF-1 or IGFBP-3 levels on the tested SNP and covariates.

Since the results in men and women are similar (none of the tests for heterogeneity of effects between men and women was significant after adjusting for multiple comparison), we pooled data from men and women to build multi-SNP predictors of IGF-1 and IGFBP-3 levels. We performed backwards stepwise selection, starting with models that contained all SNPs with univariate p-values <0.001; we retained any SNPs that had multivariable adjusted p<0.10. These models retained the design variables gender, age, cancer status, and batch. We calculated percentage of variation of IGF-1 (and IGFBP-3) that was explained by final SNPs kept in the model using the formula $(RSS_{\mathrm{cov}\ only} - RSS_{full})/RSS_{\mathrm{cov}\ only}$, where $RSS_{covonly}$ is the residual sum of squares for the model including only covariates, and RSS_{full} is residual sum of squares for the final full model including both covariates and SNPs.

To test whether the SNPs associated with circulating IGF-1 or IGFBP-3 levels were associated with risk of prostate or breast cancer, we used the SNPs retained in the final model to create separate IGF-1 and IGFBP-3 scores for each subject. We did so by taking a weighted sum of putative risk alleles (those that increased levels of IGF-1 or decreased IGFBP-3) for each subject across the set of SNPs associated with each biomarker. Each allele was weighted by its regression coefficient from the multivariable model of the biomarker on significant SNPs (see previous paragraph). We then examined the associations between the IGF-1 and IGFBP-3 scores and cancer incidence (breast cancer among women and prostate cancer among men), using a logistic regression model. This model included main effects for each of the two scores, as well as main effects for study and age (in five-year age categories).

RESULTS

Population characteristics

Table 1 shows the characteristics of the case-control samples stratified by gender. In men, prostate cancer cases had 2.0 % higher circulating IGF-1 and 3.2 % higher IGFBP-3 levels than controls (p=0.002 and 0.003 respectively), while in women, breast cancer cases and

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controls showed no significant differences in either IGF-1 or IGFBP-3 levels. Circulating levels of both IGF-1 and IGFBP-3 decreased with age ($p<10^{-4}$) and differed by cohort ($p<10^{-3}$) and gender ($p<0.01$ for IGF-1: $p<0.05$ for IGFBP-3). Age at blood draw differed by cohort ($p<10^{-4}$ for both men and women), but was similar between cancer patients and controls.

Homogeneity between men and women for the association of SNPs and circulating IGF-1/IGFBP-3

We did not detect any significant evidence of heterogeneity between men and women in the association of SNPs in IGF pathway genes with circulating levels of IGF-1 or IGFBP-3. A total of 302 SNPs were available in both the prostate cancer and breast cancer studies. The quantile-quantile plot for the *Q* test of heterogeneity did not differ from that expected under the null hypothesis (i.e. none of the associations between SNPs and circulating levels differed between men and women). No *Q* test was significant at the $\alpha=0.05$ level after adjusting for the number of SNPs tested. Due to the observed lack of heterogeneity between men and women for most SNPs, we combined the data for men and women in further analyses. We report association results for all tested SNPs in men and women separately as well as the combined sample in the Supplementary Data.

Association between SNPs and circulating IGF-1 level

In analyses of men and women combined, two SNPs in *IGF1* and four in *SSTR5* were significantly associated with circulating IGF-1 levels after adjustment for the number of independent markers tested in the pathway (nominal p-value $< 2.1 \times 10^{-4}$; Table 2). An additional SNP in *IGFALS* (rs344352) was significantly associated with IGF-1 levels when only adjusting for the number of SNPs tested within each gene.

The genomic control inflation factor for IGF-1 levels was 1.59 among men, 1.20 among women, and 1.23 in the meta-analysis combining unadjusted regression parameters for men and women. These inflation factors did not substantially change after stratifying the analyses by study and creating summary association statistics using meta-analysis, or after inverse-normal transforming residuals from a linear regression on the non-genetic covariates (including laboratory batch). The relatively high values for λ_{GC} suggest some residual systematic bias (including possible population stratification bias) that we have not adjusted for analytically in the linear regression; they might also reflect an enrichment for SNPs truly associated with IGF1 levels in these eighteen genes. To guard against false positives due to potential biases, we also calculated λ_{GC}-corrected p-values (see Methods). After λ_{GC} correction, one SNP in *IGF1* and one SNP in *SSTR5* were significantly associated with circulating IGF-1 levels (corrected p-value $< 2.1 \times 10^{-4}$).

Sensitivity analyses excluding all cases diagnosed less than two years after blood draw did not qualitatively alter these results. The *IGF1* and *SSTR5* regions still contained the five most significant SNPs. The correlation in effect estimates between the original analyses and the sensitivity analyses for the SNPs in Table 2 was over 0.98, and despite the reduced sample size rs3751830 in *SSTR5* remained pathway-wide significant.

Five SNPs remained in a multi-SNP regression model for IGF-1 levels in a backwards stepwise selection analysis beginning with 13 SNPs with $p<0.001$: rs1520220 (*IGF1*), rs3751830 (*SSTR5*), rs213656 (*SSTR5*), rs344352 (*IGFALS*), and rs3770473 (*IGFBP2/5*). These five SNPs explained 0.62% of the variation in circulating IGF-1 within our study population.

Association between SNPs and circulating IGFBP-3 levels

Six SNPs in *IGFBP3* and four in *IGFALS* were significantly associated with circulating IGFBP-3 levels after adjusting for multiple testing at the pathway level (Table 3). The λ_{GC}

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values for IGFBP-3 levels were 1.30 for men, 1.39 for women, and 1.20 for the combined analysis. All six SNPs in *IGFBP3* and two of the SNPs in *IGFALS* remained statistically significant (corrected p-value < 2.1×10^{-4}) after λ_{GC}-correction.

Sensitivity analyses excluding all cases diagnosed less than two years after blood draw did not qualitatively alter these results. The *IGFBP3* and *IGFALS* regions still contained the ten most significant SNPs, and SNPs in both of these genes remain significant at the pathway level. The correlation in effect estimates between the original analyses and the sensitivity analyses for the SNPs in Table 3 was over 0.99.

Five SNPs remained in a regression model for IGFBP-3 levels after backwards stepwise selection, starting with the 14 SNPs with p<0.001: rs2854746 (IGFBP3), rs11865665 (IGFALS), rs344352 (IGFALS), rs3770473 (IGFBP2/5), and rs10228265 (IGFBP1). These SNPs explained R^2=3.9% of the variation in circulating IGFBP-3 within our study population.

IGF-1 and IGFBP-3 predicting scores and cancer status

Using SNPs that were associated with IGF-1 or IGFBP-3, we created two separate genetic scores (see Methods section) predicting IGF-1 and IGFBP-3 levels respectively and examined their association with cancer status. No significant associations were noted between either score (IGF-1 or IGFBP-3) and either cancer. The odds ratio (OR) associated with a one standard deviation increase in the IGF-1 score was 0.99 (0.93, 1.05) for prostate cancer and 1.01 (0.96, 1.07) for breast cancer. The OR associated with a one standard deviation increase in the IGFBP-3 score was 0.98 (0.93, 1.03) for prostate cancer and 1.00 (0.94, 1.05) for breast cancer.

DISCUSSION

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This large, comprehensive study identified novel associations between common SNPs in *SSTR5* and circulating IGF-1 levels and between common SNPs in *IGFALS* and circulating IGFBP-3 levels. We also replicated previously-reported associations between SNPs in *IGF1* and IGF-1 levels and between SNPs in the *IGFBP3/BP1* region and IGFBP3 levels (8–9,12).

IGFALS gene

In our analyses we found four tagging SNPs in the *IGFALS* gene that were statistically significantly associated with circulating IGFBP-3 after multiple testing adjustment at the pathway level; one of these SNPs was also statistically significantly associated with IGF-1 levels after adjustment for multiple testing at the gene level. The protein product of this gene (a liver-derived, GH-dependent, 85-kD glycoprotein) is a key component of the 150-kD three-part (ternary) IGF circulating complex (46). More than 80% of IGF-1 circulates as a ternary complex, comprised of one molecule each of IGF-1, IGFBP-3 or 5, and IGFALS (47–48). The half-life of IGF-1 carried as this ternary complex is 12 h, much longer than that of binary, bound IGF (30–90 min) or free, unbound IGF-1 (10 min) (49). Our study suggests that common genetic variants may also contribute to the variation of circulating IGF-1 and IGFBP-3. Additional studies will be needed to identity the precise causal variant(s) in this region.

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SSTR5 gene

We found four SNPs in *SSTR5* that were significantly associated with circulating IGF-1 levels after adjustment for the number of independent tests at the pathway level. A previous (15) study in the EPIC cohort examined 4 SNPs within the *SSTR5* gene and reported one synonymous SNP rs642249 in exon 1 associated with IGF-1 level. Another study within the Swedish CAPS project examined five SNPs in the *SSTR5* gene and showed strongly significant associations between the missense SNP rs4988483 and reduced circulating levels of both IGF1 and IGFBP3 (13). Data on linkage disequilibrium between rs642249 and the SNPs genotyped

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in this study are unavailable. Data from the 1000 Genomes Project CEU panel suggest the linkage disequilibrium between rs498843 and SNPs in this study is low (r^2<0.05). Thus it is difficult to assess whether the previous results are consistent with those we present here. The *SSTR5* gene encodes one of five somatostatin receptors; these structurally related proteins are members of the seven transmembrane-spanning family of G protein-coupled receptors. Somatostatin binds with these receptors to exert its biological functions, including inhibition of GH secretion from the pituitary (50). It is possible that common SNPs located within or near *SSTR5* influence its expression or its affinity with SST and thereby inhibit GH production, which eventually affects the production of IGF-1.

IGF1 gene

We found two SNPs in *IGF1* (rs1520220 and rs10735380) that were statistically significantly associated with IGF-1 levels in the combined sample of men and women after adjusting for multiple testing at that pathway level. These results are similar to previous analyses of BPC3 data conducted in men and women seperately (16–17) These observations are consistent with reports by Tamimi et al. (51) and Al-Zahrani et al. (9). Tamimi et al. found the SNP rs1520220 was associated with mammographic density in women; this association may be mediated through this SNP's effect on IGF-1 expression. Al-Zahrani et al. also found that the minor allele of rs1520220 was significantly associated with an increase in circulating IGF-1 in women.

More than 10 studies have examined possible associations between circulating IGF-1 levels and a simple sequence length polymorphism (CA)n 1 kb upstream of the *IGF1* gene, but the results have been inconsistent (11). We did not genotype *IGF1* (CA)n, but according to a previous report, the less common repeat length for this polymorphism is in LD with the minor alleles of rs7965399 and rs35767 (17). We did examine these two SNPs, and one was significant after adjusting for multiple comparisons at the gene level (rs35767: p=0.2 in women, 0.0028 in men, 0.0034 in combined). In a meta-analysis combining nearly 1300 Swedish men and 4 other studies, two tagging SNPs (rs6220 and rs7136446) in the 3' region of *IGF1* gene were reported to be associated with circulating IGF-1 (12). Although we did not genotype rs7136446, we could impute genotypes for this SNP (the estimated correlation between the imputed and true genotypes was greater than 0.67). We found a nominally significant association between the minor allele of rs7136446 and increasing IGF-1 levels (p=0.009 for men and women combined). We could not impute rs6220, because it has not been genotyped in the HapMap CEU reference panel.

Taken together, the previous findings and our study suggest that common genetic variation in the *IGF1* gene influences circulating IGF-1 levels. The SNP rs1520220 is consistently associated with circulating IGF-1, although the functional mechanism underlying this association still needs to be explored.

IGFBP3 gene

The positive association of the SNP C-202A (rs2854744) in the promoter region of the *IGFBP3* gene with circulating IGFBP-3 levels has been noted consistently in at least eleven studies, including two recent reports from the BPC3 group in men and women (9–10,16,27, 52–58). An *in vitro* study has suggested the A allele of this SNP had higher promoter activity (54). The BPC3 genotyped 16 SNPs in the IGFBP1/3 gene region and their flanking area (8 SNPs in each gene region), among which six SNPs in the *IGFBP3* region were significant in our analyses after multiple comparison adjustment at the pathway level, with p values ranging from 4.50×10^{-83} (rs2854746) to 1.20×10^{-6} (rs2270628) (Table 3); this is consistent with the two BPC3 reports (16–17) As noted by multiple studies (16,27,59) the most significant SNP was rs2854746, a nonsynonymous polymorphism in exon 1 (Gly32Ala), rather than the

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extensively reported promoter polymorphism rs2854744 (A-202C). Although the exact functional SNP influencing the IGFBP-3 levels remains to be identified, our analyses together with previous reports suggest the existence of common variants in the *IGFBP3* gene that influence circulating IGFBP-3 levels.

IGF-1 and IGFBP-3 predicting scores and cancer status

Although both IGF-1 and IGFBP-3 levels were associated with prostate cancer in our study, no association was observed between either (IGF-1 or IGFBP-3) score predicted by SNP risk alleles and prostate (or breast) cancer. This is consistent with the fact that the SNPs used to create these scores explain only a small percentage of the variation in IGF-1 (<0.7%) or IGFBP-3 levels (<4%). The finding of no association between predicting scores and breast cancer is consistent with the null association between circulating IGF-1 and IGFBP-3 and breast cancer risk in our data. Given that twin studies show that up to 60% IGF-1 and IGFBP-3 in adults are explained by heritable factors (7–8,60), the reason behind the small percentages of IGF-1 and IGFBP-3 explained by these pathway genes needs to be explored.

Strengths and limitations

To our knowledge, this is the most comprehensive candidate gene study to examine the genetic factors affecting circulating IGF-1 and IGFBP-3 in terms of 1) the numbers of genes (18), 2) the coverage of each gene (SNPs were chosen to tag both SNPs in the HapMap CEU panel and exonic variants detected by resequencing), and 3) the number of subjects. With germline DNA samples from over 11,000 individuals, we have 99%, 97%, 92%, and 51% power to detect SNPs that can explain 0.3%, 0.25%, 0.15%, and 0.1% (respectively) of IGF-1 (or IGFBP-3) variation at the 0.001 significance level with 2-sided tests. The strict multiple comparison adjustment at the pathway level and the conservative use of genomic control decreases the possibility of false positives. Because ours is a multi-center study, we encountered challenges such as differences in laboratory assays for biomarker measurement and differences in lifestyle and environmental factors that might contribute to IGF-1 (or IGFBP-3) variation. To solve this problem, we adjusted for age and assay batch,. Given the relatively large genomic control inflation factor for the SNP-IGF-1 association among men, we cannot rule out systematic bias such as population stratification; however, after we corrected for genomic control, the top SNPs were still significant.

Conclusion

In summary, our study suggests that common genetic variation in the *IGF1* and *SSTR5* genes affects circulating IGF-1 levels, and that the same may be true for variation in the *IGFALS* gene. In addition, common genetic variation in the *IGFBP3* and *IGFALS* genes may influence the concentration of circulating IGFBP-3. Common genetic variation in the other 14 genes in the IGF signaling pathway that we studied had no important impact on circulating IGF-1 or IGFBP-3 levels. Common genetic variation in the pathway accounts for only a small percentage of circulating levels of these biomarkers among Caucasians (0.62% for IGF-1 and 3.9% for IGFBP-3). Other factors such as rare variants in these genes, structural changes in these genes, genetic variation outside of the regions we studied, and gene-environmental interactions, along with lifestyle and environmental exposures, may make their own contributions to IGF-1 and IGFBP-3 variation in population.

Supplementary Material

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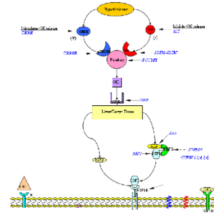

Figure 1. Overview of IGF signaling pathway upstream of the IGF receptors

Overview of insulin-like growth factor pathway upstream of IGF1R The hypothalamus regulates the release of growth hormone (GH) by either stimulating the pituitary gland with growth hormone-releasing hormone (GHRH) or inhibiting it with somatostatin (SST). Circulating IGFs are mainly produced by the liver after stimulation by GH. The insulin-like growth factor 1 receptor (IGF1-R) binds either insulin-like growth factor 1 (IGF1) or insulin-like growth factor 2 (IGF2). Most of circulating IGF1 is bound in a ternary complex with insulin-like growth factor binding protein 3 (IGFBP3) and the acid-labile subunit (ALS). In the ternary complex, IGF1 cannot cross the endothelial cell barrier; its half-life in circulation is prolonged and its ability to activate IGFR1 is reduced. The binding of IGFs to IGF1R stimulates the receptor's tyrosine kinase activity, which activates downstream signaling pathways, including the PI3K-AKT-TOR and RAF-MAPK systems. These downstream networks stimulate angiogenesis and cell proliferation and survival, and inhibit apoptosis. The insulin-like growth factor 2 receptor (IGF2R) binds only IGF-2 and does not activate intracellular signaling pathways. The insulin receptor (IR) can bind either insulin (INS) or IGF-I. **Other abbreviations:** GHRHR, growth hormone releasing hormone receptor; SSTR, somatostatin receptor; GHR, growth hormone receptor; POU1F1, pituitary-specific positive transcription factor 1. **Text color scheme:** The blue text signifies genes analyzed.

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Table 1

Distributions of circulating IGF-1 and IGFBP-3 levels and age at blood draw, by study

Cohort	Gender	($N_{case}/N_{control}$)	ln(IGF-1), in ng/ml mean (SD)		ln(IGFBP-3), in ng/ml mean (SD)		Age at blood draw mean (SD)		Mean time from blood draw to diagnosis (cases)
			cases	controls	cases	controls	cases	controls	
ATBC	men	89/226	4.96(0.35)	4.93(0.36)	7.81(0.28)	7.76(0.28)	59.5(4.9)	56.9(4.9)	9.5(2.0)
EPIC	men	617/628	5.12(0.37)	5.08(0.42)	8.22(0.19)	8.21(0.20)	61.4(6.1)	61.5(6.2)	3.4(2.0)
HPFS	men	653/646	5.19(0.31)	5.14(0.32)	8.11(0.29)	8.07(0.31)	65.7(7.5)	65.6(7.4)	3.3(1.8)
MEC	men	3/63	5.17(0.50)	5.13(0.43)	8.00(0.35)	7.87(0.33)	67.0(4.6)	67.7(6.9)	1.1(1.0)
PHS	men	594/521	5.18(0.38)	5.17(0.37)	8.01(0.24)	8.01(0.26)	60.2(7.8)	61.0(7.6)	8.7(3.4)
PLCO	men	723/845	5.28(0.41)	5.25(0.41)	8.41(0.24)	8.40(0.24)	65.6(4.8)	65.3(4.8)	2.8(1.4)
Total	men	2679/2929	5.18(0.39)	5.15(0.40)	8.17(0.30)	8.15(0.33)			
			P*=0.002		P=0.003		P=0.34		
EPIC	women	1065/2097	5.43(0.34)	5.41(0.33)	8.12(0.36)	8.1(0.36)	54.7(8.7)	54.7(8.7)	2.4(1.9)
MEC	women	1/72	5.45(−)	4.91(0.46)	8.13(−)	7.89(0.36)	57.6(−)	67.0(7.2)	−0.8 (−)**
NHS	women	326/400	5.34(0.35)	5.32(0.33)	8.44(0.19)	8.44(0.18)	63.7(5.2)	63.5(5.2)	3.9(2.2)
PLCO	women	696/894	5.01(0.38)	5.01(0.36)	8.4(0.24)	8.41(0.23)	56.5(7.0)	57.7(7.0)	4.0(2.5)
Total	women	2088/3463	5.27(0.41)	5.29(0.39)	8.27(0.34)	8.21(0.35)			
			P=0.43		P=0.18		P=0.21		

* P-value of association between case/control status and population characteristic after adjusting for cohort/gender, and age of blood draw for ln (IGF-1) and ln (IGFBP-3). The p values of the associations between cohort and each population characteristic were all < 0.001 for both men and women after adjusting for case-control status, and age of blood draw for ln (IGF-1) and ln (IGFBP-3).

** Except for this one prevalent breast cancer case, all IGF-1 and IGFBP3- measurements were made using blood samples collected prior to cancer diagnosis.

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Table 2

Associations between single SNPs and circulating IGF-1[†]

SNP	Gene	Sample size		WT>VT	MAF		% change of IGF-1 per minor alleles (95%CI)			P		
		w	m		w	m	W	m	combined	w	m	combined
rs1520220	IGF1	5533	5379	C>G	0.18	0.19	2.7(1.1,4.4)	2.4(0.6,4.3)	2.6(1.4,3.4)	6.0×10^{-4}	6.7×10−3	1.4×10^{-5}
rs3751830	SSTR5	5533	5047	C>T	0.41	0.41	−2.1(−3.4,−0.7)	−2.1(−3.4, −0.7)	−2.1(−3.0,−5.4)	2.7×10^{-3}	4.8×10^{-3}	3.8×10^{-5}
rs197057	SSTR5	5393	3937	G>C	0.47	0.46	1.8(0.6,3)	2.3(0.7,3.9)	2.0(1.0,2.5)	5.3×10^{-3}	6.05×10^{-3}	1.0×10^{-4}
rs174643*	SSTR5	3875	5047	A>G	0.44	0.45	2.5(1.1,3.9)	1.5(0.1,2.9)	2.0(1.0,2.5)	6.2×10^{-4}	3.82×10^{-2}	1.0×10^{-4}
rs197056	SSTR5	3947	5047	G>A	0.37	0.38	2.4(1,3.8)	1.6(0.2,3)	2.0(1.0,2.5)	1.1×10^{-3}	2.64×10^{-2}	1.1×10^{-4}
rs10735380	IGF1	5533	5379	A>G	0.26	0.28	2.1(0.7,3.5)	1.7(0.1,3.3)	1.9(0.9,2.3)	2.7×10^{-3}	2.45×10^{-2}	1.9×10^{-4}

*These SNPs were successfully genotyped in female cohorts, but failed genotyping and had to be imputed in male cohorts; all the other SNPs were successfully genotyped in both females and males.

WTVT: wild type (common) allele>variant (minor) allele. MAF: minor allele frequency.

†Only SNPs that were significant in combined analyses after multiple-testing correction at pathway level (p value $< 2.1 \times 10^{-4}$)

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Table 3

Associations between single SNPs and circulating IGFBP-3[†]

SNP	Gene	Sample size		WT>VT	MAF		% change of IGFBP-3 per minor alleles(95%CI)			P		
		w	m		w	m	W	m	combined	w	m	combined
rs2854746	IGFBP3	5209	5199	G>C	0.43	0.4	5.9(5,6.7)	6.4(5.6,7.2)	6.1(5.5,11.3)	4.6×10^{-41}	3.5×10^{-54}	4.5×10^{-83}
rs2960436	IGFBP3	5332	5284	G>A	0.49	0.46	5(4.2,5.8)	5.5(4.7,6.4)	5.3(4.7,9.8)	4.4×10^{-32}	1.6×10^{-41}	3.6×10^{-66}
rs2854744	IGFBP3	5310	5291	G>T	0.48	0.46	5.1(4.3,6)	5.5(4.7,6.4)	5.3(4.7,9.8)	5.2×10^{-31}	1.6×10^{-41}	4.4×10^{-65}
rs3110697	IGFBP3	5420	5357	G>A	0.42	0.42	−3.6(−4.4,−2.9)	−3.9(−4.7,−3.2)	−3.7(−4.3,−8)	6.1×10^{-18}	1.5×10^{-23}	2.8×10^{-35}
rs2132570	IGFBP3	5278	5208	G>T	0.21	0.22	−2.6(−3.5,−1.6)	−2.6(−3.5,−1.6)	−2.6(−3.3,−6)	1.2×10^{-6}	7.8×10^{-7}	4.2×10^{-12}
rs2270628	IGFBP3	5420	5357	C>T	0.18	0.2	−1.6(−2.5,−0.6)	−2.1(−3,−1.1)	−1.9(−2.6,−4.7)	3.6×10^{-3}	8.2×10^{-5}	1.2×10^{-06}
rs11865665*	IGFALS	5420	5067	A>G	0.07	0.07	2.4(0.8,4)	3.3(1.4,5.1)	2.8(1.6,3.9)	3.7×10^{-3}	3.8×10^{-4}	5.2×10^{-06}
rs344352	IGFALS	5343	5018	C>G	0.28	0.3	−1.1(−2.1,−0.1)	−2(−2.9,−1)	−1.5(−2.1, −3.7)	2.0×10^{-2}	5.4×10^{-5}	7.9×10^{-06}
rs17559	IGFALS	5420	5067	G>A	0.09	0.09	1.6(0,3.2)	2.7(1.1,4.4)	2.1(1.1,2.7)	3.3×10^{-2}	5.4×10^{-4}	8.2×10^{-05}
rs1178436	IGFALS	5420	5067	C>T	0.15	0.16	−1(−2.2,0.2)	−2.2(−3.3,−1)	−1.6(−2.4,−4.2)	9.6×10^{-2}	2.3×10^{-4}	1.7×10^{-04}

*These SNPs were successfully genotyped in female cohorts, but failed genotyping and had to be imputed in male cohorts; all the other SNPs were successfully genotyped in both females and males.

WTVT: wild type (common) allele>variant (minor) allele. MAF: minor allele frequency.

†Only SNPs that were significant in combined analyses after multiple-testing correction at pathway level (p value $< 2.1 \times 10^{-4}$)