

A genome-wide association study identifies novel loci associated with circulating IGF-I and IGFBP-3

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Insulin-like growth factor-I (IGF-I) and insulin-like growth factor-binding protein-3 (IGFBP-3) are involved in cell replication, proliferation, differentiation, protein synthesis, carbohydrate homeostasis and bone metabolism. Circulating IGF-I and IGFBP-3 concentrations predict anthropometric traits and risk of cancer and cardiovascular disease. In a genome-wide association study of 10 280 middle-aged and older men and women from four community-based cohort studies, we confirmed a known association of single nucleotide polymorphisms in the *IGFBP3* gene region on chromosome 7p12.3 with IGFBP-3 concentrations using a significance threshold of $P < 5 \times 10^{-8}$ ($P = 3.3 \times 10^{-101}$). Furthermore, the same *IGFBP3* gene locus (e.g. rs11977526) that was associated with IGFBP-3 concentrations was also associated with the opposite direction of effect, with IGF-I concentration after adjustment for IGFBP-3 concentration ($P = 1.9 \times 10^{-26}$). A novel and independent locus on chromosome 7p12.3 (rs700752) had genome-wide significant associations with higher IGFBP-3 ($P = 4.4 \times 10^{-21}$) and higher IGF-I ($P = 4.9 \times 10^{-9}$) concentrations; when the two

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measurements were adjusted for one another, the IGF-I association was attenuated but the IGFBP-3 association was not. Two additional loci demonstrated genome-wide significant associations with IGFBP-3 concentration (rs1065656, chromosome 16p13.3, $P = 1.2 \times 10^{-11}$, *IGFALS*, a confirmatory finding; and rs4234798, chromosome 4p16.1, $P = 4.5 \times 10^{-10}$, *SORCS2*, a novel finding). Together, the four genome-wide significant loci explained 6.5% of the population variation in IGFBP-3 concentration. Furthermore, we observed a borderline statistically significant association between IGF-I concentration and *FOXO3* (rs2153960, chromosome 6q21, $P = 5.1 \times 10^{-7}$), a locus associated with longevity. These genetic loci deserve further investigation to elucidate the biological basis for the observed associations and clarify their possible role in IGF-mediated regulation of cell growth and metabolism.

INTRODUCTION

Insulin-like growth factors (IGFs) constitute an evolutionarily conserved system of peptides that has diverse functions throughout embryonic development, growth and adulthood. IGF-I, which is carried in the circulation by IGF-binding protein-3 (IGFBP-3), is synthesized by the liver and by most other tissues of the body upon stimulation by growth hormone (GH) and is a central mediator of metabolic, endocrine and anabolic effects of GH. IGF-I promotes cell proliferation and differentiation, inhibits apoptosis and has insulin-like metabolic effects (1). A family of six IGFBPs bind with high affinity to IGF-I and may inhibit or potentiate interaction of IGF-I with its receptors, which include the IGF-I receptor and the insulin receptor. In addition to its effects on IGF-I bioavailability, IGFBP-3 has been shown to have intrinsic antiproliferative and proapoptotic activity in experimental studies (2–4). In population-based studies, circulating IGF-I and IGFBP-3 concentrations as well as genes for IGF proteins, receptors and downstream signaling molecules (e.g. IGF-I receptor) have been associated with longevity (5), cancer (6,7) and common chronic diseases (8–17).

Prior work has shown high heritability for blood IGF-I (~40–60%) and IGFBP-3 concentrations (60%) (18–20). We conducted a genome-wide association (GWA) study to identify single nucleotide polymorphisms (SNPs) associated with circulating IGF-I and IGFBP-3 concentrations among 10 280 middle-aged to older adults of European origin from four population-based cohorts.

RESULTS

Included in the analyses were 10 280 men and women, comprising 1712 participants in the Cardiovascular Health Study (CHS), 3507 participants in the Framingham Heart Study (FHS), 1607 participants in the Cooperative Research in the Region of Augsburg (KORA) study and 3454 participants in the Study of Health in Pomerania (SHIP). Characteristics are presented in Table 1, with additional details in Supplementary Material, Table S1.

SNPs associated with IGFBP-3 concentrations

Four SNPs had genome-wide significant associations with IGFBP-3 concentrations (Table 2, Q–Q and Manhattan plots appear in Supplementary Material, Figure S1, cohort-specific results appear in Supplementary Material, Table S2). These

four genome-wide significant SNPs were: rs11977526 on chromosome 7p12.3, minor allele frequency (MAF) = 0.41, $P = 3.3 \times 10^{-101}$ (Fig. 1, top panel); rs700752 on chromosome 7p12.3, MAF = 0.35, $P = 4.4 \times 10^{-21}$ (Fig. 2, bottom panel); rs4234798 on chromosome 4p16.1, MAF = 0.39, $P = 4.5 \times 10^{-10}$ (Fig. 3) and rs1065656 on chromosome 16p13.3, MAF = 0.32, $P = 1.2 \times 10^{-11}$ (Fig. 4). Within each study cohort, the effect size for rs11977526 was larger than that for the other genome-wide significant SNPs. Together, these four genome-wide significant SNPs explained 6.5% of the population variation in IGFBP-3 concentrations. Within the four cohorts, r^2 values ranged from 5.4 to 7.7% of intra-individual variance explained (Supplementary Material, Table S3).

The SNP that was most strongly associated with IGFBP-3 concentrations, rs11977526, is in the *IGFBP3* region on chromosome 7p12.3, which is known to be associated with circulating IGFBP-3 (21–23). In the SHIP cohort, we individually genotyped rs2854746 (non-synonymous, exon 1, Gly32Ala), which appears to be the strongest SNP signal in the region (23) and which has strong linkage disequilibrium (LD) with rs11977526 ($r^2 = 0.89$). Figure 1, bottom panel, presents the regional plot for GWA results, including rs11977526, in the SHIP cohort. After adjustment for rs2854746, we observed no significant independent association between rs11977526 and IGFBP-3 concentration ($P = 0.13$). In the same model, rs2854746 remained associated with IGFBP-3 concentration after adjustment for rs11977526 ($P = 1.1 \times 10^{-5}$). The other genome-wide significant SNP on chromosome 7p12.3 (rs700752) remained associated with IGFBP-3 concentrations after adjustment for either rs11977526 ($P = 6.4 \times 10^{-8}$) or rs2854746 ($P = 1.4 \times 10^{-7}$).

Another SNP associated with IGFBP-3 concentrations, rs1065656, is in a region near *IGFALS* that was recently reported by Gu *et al.* (24) to associate with IGFBP-3 levels. We conducted conditional analyses that examined rs1065656 in combination with the significant SNPs reported by Gu *et al.* (rs11865665, rs17559, rs1178463 and rs344352). These analyses suggested that rs1065656, rs1178463 and rs344352 represent one IGFBP-3-related locus in the *IGFALS* region, and rs11865665 and rs17559 probably represent a second independently associated locus.

SNPs associated with IGF-I concentrations

Analyses of SNPs associated with IGF-I concentrations appear in Table 2 (see Q–Q and Manhattan plots in Supplementary

Table 1. Characteristics of 10 280 individuals and genotyping methods for the four cohorts included in the meta-analysis

	Cardiovascular Health Study (CHS)	Framingham Heart Study (FHS)	Cooperative Research in the Region of Augsburg (KORA)	Study of Health in Pomerania (SHIP)
<i>N</i> ^a	1712	3507	1607	3454
Males, %	36	46	48	49
Age [mean (SD)], years	71.5 (4.8)	39.9 (8.7)	60.0 (8.7)	47.9 (15.7)
Body mass index (mean (sd)), kg/m ²	26.1 (4.3)	26.8 (5.5)	27.8 (4.6)	27.1 (4.7)
IGF-I, median (Q1, Q3)	153.3 (115.9, 195.9)	124.5 (101.3, 152.2)	128.0 (101.0, 157.0)	136.0 (105.1, 173.7)
IGFBP-3, median (Q1, Q3)	3846 (3261, 4474)	2960 (2273, 3824)	3405 (2870, 3972)	1918 (1596, 2210)
Genotyping center	Cedars-Sinai Medical Center	Affymetrix	HMGU, Neuherberg, Germany	Affymetrix
Genotyping platform and SNP panel	Illumina 370CNV	Affymetrix 550K	Affymetrix 6.0	Affymetrix 6.0
Genotyping calling algorithm	Illumina BeadStudio	BRLMM	Birdseed2	Birdseed2
Average call rate	95%	98%	98%	98%
Imputation software	BIMBAM10 v0.99	MACH	IMPUTE v0.4.2	IMPUTE v0.5.0
Number of SNPs in data set after QC	306 655	378 163	869 339	869 224
Number of SNPs in imputed data set	2 332 667	2 543 887	2 743 205	2 748 910

^aShown are sample sizes for analyses of IGF-I concentrations. For analyses of IGFBP-3 concentrations, sample sizes were 1465 for CHS, 3507 for FHS, 1592 for KORA and 3454 for SHIP.

Table 2. Loci associated with IGF-I and IGFBP-3 concentrations at the genome-wide significant ($P < 5 \times 10^{-8}$) and $P < 1 \times 10^{-6}$ level

	Chr	Position	A1	A2	F1	IGF-I, <i>P</i> -value	IGFBP-3, <i>P</i> -value	Nearby genes	Gene distance
rs11977526	7	45,974,635	a	g	0.41	n.s.	3.3×10^{-101}	<i>IGFBP3</i>	47 239
rs700752	7	46,720,078	c	G	0.35	4.9×10^{-9}	4.4×10^{-21}	<i>TNS3</i>	561 198
rs4234798	4	7,270,834	t	g	0.39	n.s.	4.5×10^{-10}	<i>SORCS2</i>	0
rs1065656	16	1,778,837	c	g	0.32	n.s.	1.2×10^{-11}	<i>NUBP2, IGFALS</i>	0
rs7780564	7	7,849,848	a	C	0.45	3.9×10^{-7}	n.s.	<i>RPA3</i>	125 085
rs1245541	10	73,519,645	a	G	0.39	5.0×10^{-7}	n.s.	<i>SPOCK2</i>	872
rs2153960	6	109,094,877	A	g	0.69	5.1×10^{-7}	n.s.	<i>FOXO3</i>	0

Chr, chromosome; A1, allele 1; A2, allele 2; F1, frequency of allele 1; n.s., not significant. The alleles indicated by bold italicized text were associated with higher levels of circulating IGFBP-3, and the alleles indicated by upper case letters were associated with higher levels of circulating IGF-I.

Material, Fig. S2, and cohort-specific results in Supplementary Material, Table S2). rs700752 was not only associated with IGFBP-3 concentrations but was also associated with IGF-I concentrations ($P = 4.9 \times 10^{-9}$) (Fig. 2, top panel). We constructed models that examined rs700752 as a predictor of IGFBP-3 concentrations while adjusting for IGF-I concentrations, and that examined rs700752 as a predictor of IGF-I concentrations while adjusting for IGFBP-3 concentrations. These analyses showed that rs700752 remained significantly associated with IGFBP-3 concentrations after adjusting for IGF-I concentrations (meta-analysis $P = 1.4 \times 10^{-15}$), but the association of rs700752 with IGF-I concentrations was attenuated (meta-analysis $P = 0.038$) after adjustment for IGFBP-3 concentrations (Supplementary Material, Table S4).

In analyses that were not adjusted for IGFBP-3 concentrations, rs700752 was the only SNP associated with IGF-I concentration at the level of genome-wide significance. Three additional SNPs achieved $P < 10^{-6}$ in relation to IGF-I concentrations (Table 2): rs2153960 on chromosome 6q21, MAF = 0.31, $P = 5.1 \times 10^{-7}$ (Supplementary Material, Fig. S3); rs1245541 on chromosome 10q22.1, MAF = 0.39,

$P = 5.0 \times 10^{-7}$; rs7780564 on chromosome 7p21.3, MAF = 0.45, $P = 3.9 \times 10^{-7}$.

SNPs associated with IGF-I concentration, after adjustment for IGFBP-3 concentration

Analyses of IGF-I were repeated after adjustment for IGFBP-3 (see Q-Q and Manhattan plots in Supplementary Material, Fig. S4). While SNPs in the *IGFBP3* gene were not associated with IGF-I concentrations in our initial analyses, we found a genome-wide significant association of the *IGFBP3* region with IGF-I in analyses that adjusted for IGFBP-3 concentrations. The *IGFBP3* SNP most strongly associated with IGF-I concentration after adjustment for IGFBP-3 concentration was rs1496499 ($P = 3.8 \times 10^{-27}$) (Fig. 5). This SNP had no significant association with IGF-I in analyses that were adjusted only for age and sex ($P = 6.7 \times 10^{-2}$). The SNPs that were associated with higher IGFBP-3 levels were associated with lower IGFBP-3-adjusted IGF-I levels. For example, rs11977526 was the *IGFBP3* SNP most strongly associated with IGFBP-3 concentrations (Table 2). This SNP

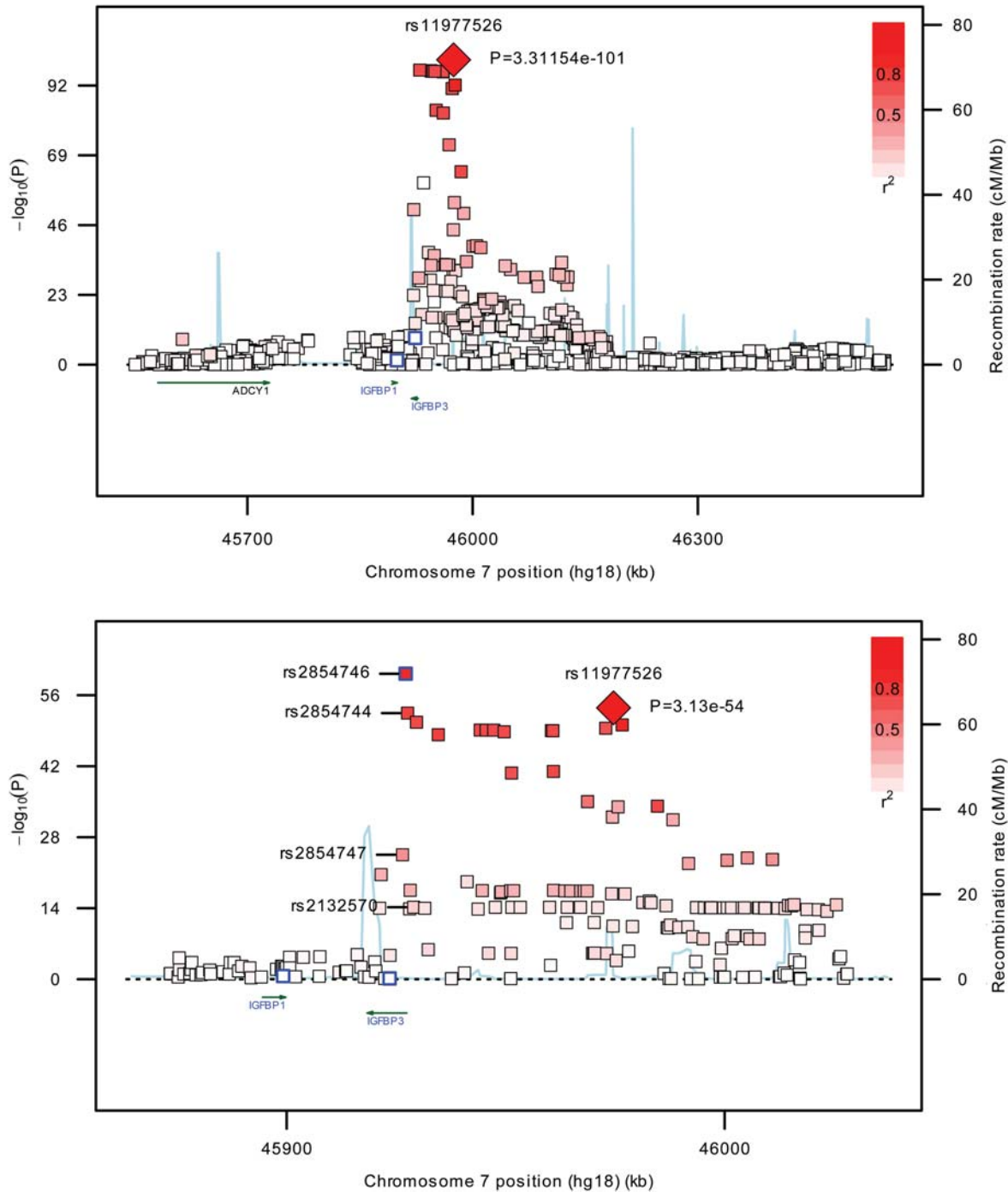


Figure 1. Regional plot (IGFBP-3) for rs11977526 on chromosome 7. The diamond indicates rs11977526, the SNP with the lowest P for association with IGFBP-3 concentration. Red shading indicates LD measure (r^2) with rs11977526. Non-synonymous SNPs in *IGFBP1* and *IGFBP3* are marked in blue. The top panel shows the overall four-cohort meta-analysis. The bottom panel indicates rs11977526, the non-synonymous *IGFBP3* exon 1 SNP rs2854746 and other neighboring SNPs measured or imputed in the SHIP cohort. Red shading indicates LD with rs11977526. The left y-axis indicates P for association with IGFBP-3 concentration, and the right y-axis indicates recombination rate.

was also associated with the opposite direction of effect, with IGF-I concentration after adjustment for IGFBP-3 concentration ($P = 1.9 \times 10^{-26}$). The analyses of IGFBP-3-adjusted IGF-I concentrations also confirmed the presence of borderline-significant associations between IGF-I and rs2153960 ($P = 4.6 \times 10^{-5}$), rs1245541 ($P = 1.6 \times 10^{-6}$)

and rs7780564 ($P = 4.4 \times 10^{-6}$); these results recapitulate the associations that appear in Table 2, which were not IGFBP-3 adjusted. As noted above, in age- and sex-adjusted analyses, rs700752 had genome-wide significant associations with both IGF-I and IGFBP-3 concentrations, but rs700752 remained associated only with IGFBP-3 (and not with

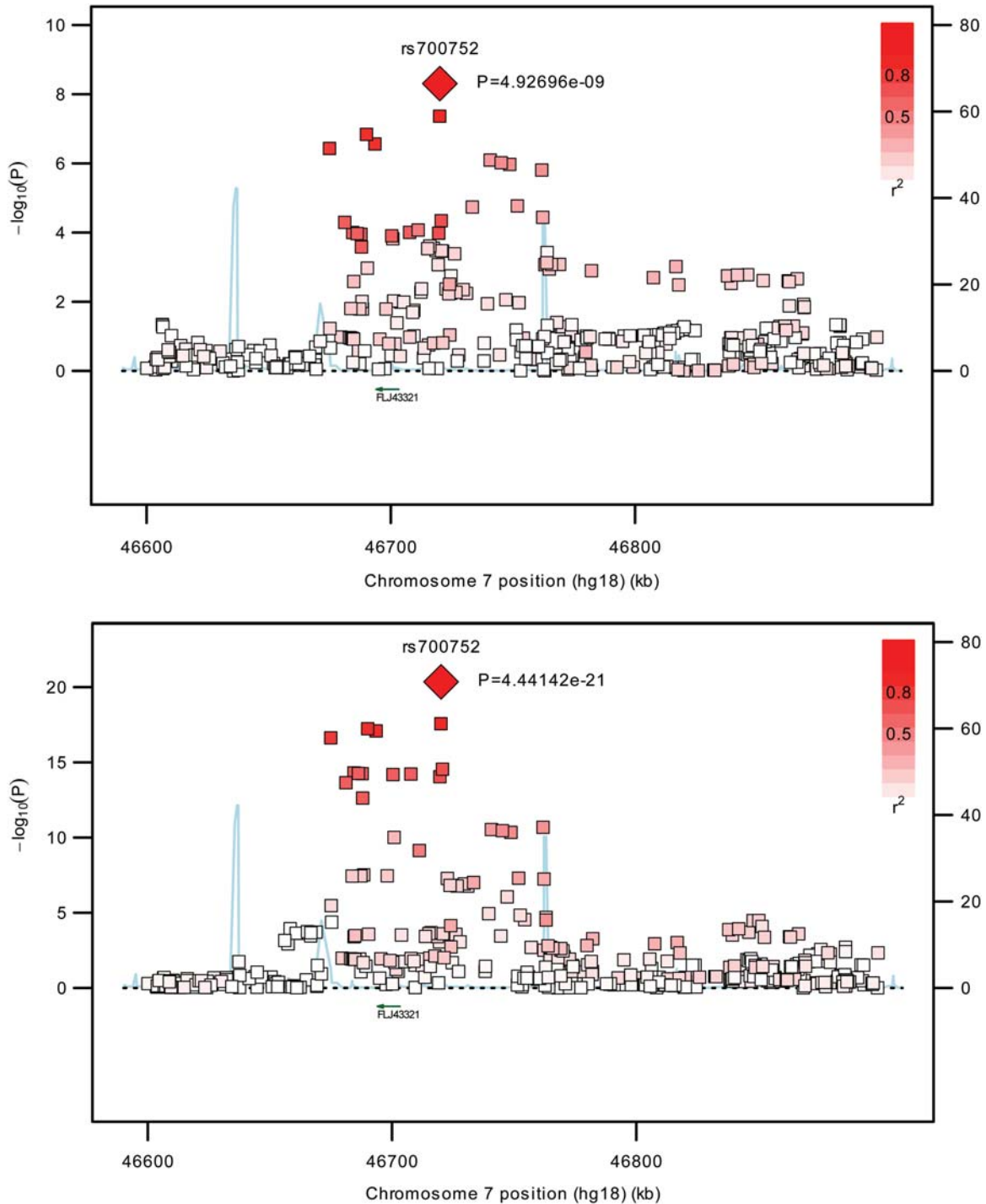


Figure 2. Regional plot (IGF-I and IGFBP-3) for rs700752 on chromosome 7. The diamond indicates rs700752, the SNP with the lowest P for association with IGF-I (top panel) and IGFBP-3 (bottom panel) concentrations. Red shading indicates LD measure (r^2) with rs700752. The left y -axis indicates P for association with IGFBP-3 or IGF-I concentration, and the right y -axis indicates recombination rate.

IGF-I) when the two analytes were mutually adjusted for one another (see Supplementary Material, Table S4). No other significant or borderline-significant SNPs were identified in analyses of IGFBP-3-adjusted IGF-I concentrations.

Sensitivity analyses

Further analyses addressed the possible influence of laboratory assay methodology on study results. In the SHIP cohort, IGF-I

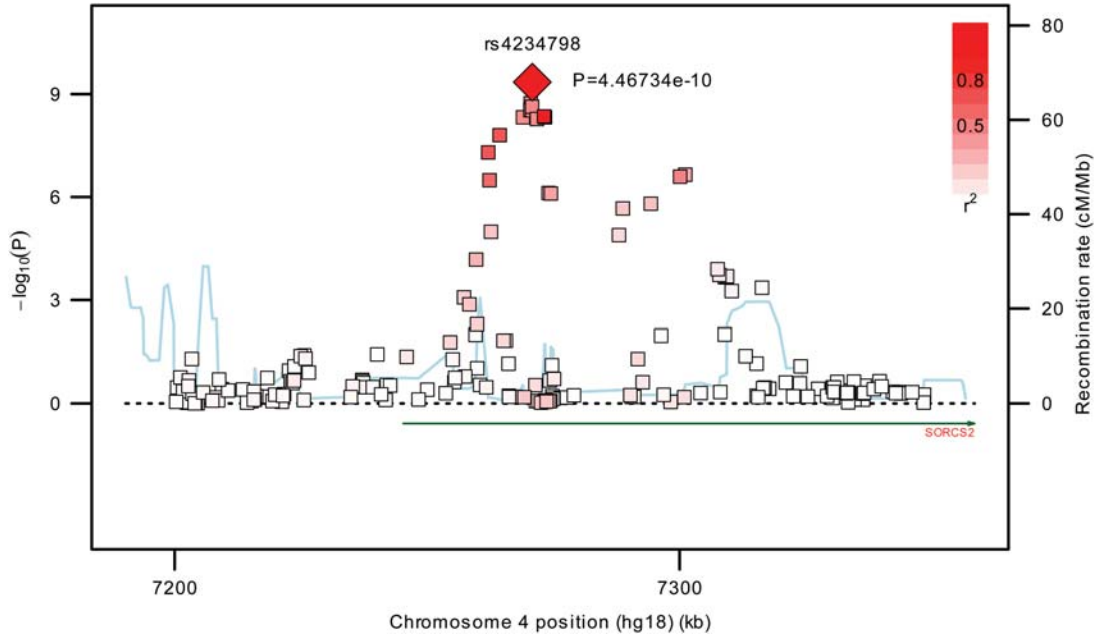


Figure 3. Regional plot (IGFBP-3) for rs4234798 on chromosome 4. The diamond indicates rs4234798, the SNP with the lowest P for association with IGFBP-3 concentration. Red shading indicates LD measure (r^2) with rs4234798. Red text indicates that rs4234798 lies within the *SORCS2* gene. The left y-axis indicates P for association with IGFBP-3 concentration, and the right y-axis indicates recombination rate.

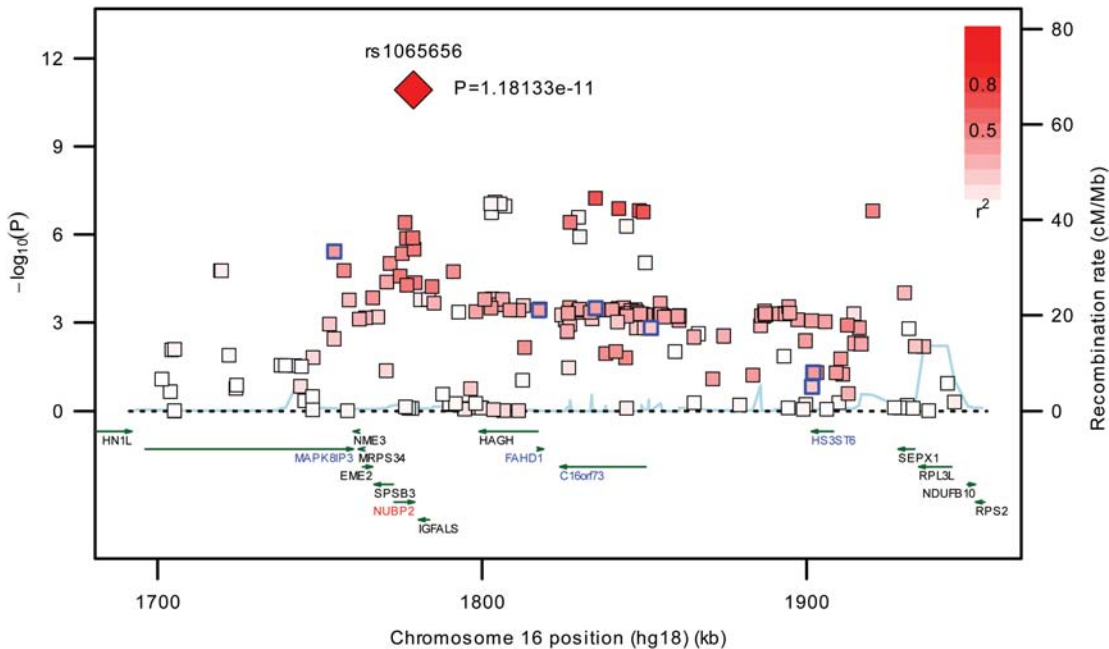


Figure 4. Regional plot (IGFBP-3) for rs1065656 on chromosome 16. The diamond indicates rs1065656, the SNP with the lowest P for association with IGFBP-3 concentration. Red shading indicates LD measure (r^2) with rs1065656. Non-synonymous SNPs and corresponding genes are marked in blue. The left y-axis indicates P for association with IGFBP-3 concentration, and the right y-axis indicates recombination rate.

and IGFBP-3 measurements were repeated at two study visits using different assay methods (see Materials and Methods). A sensitivity analysis suggested that the two different laboratory assays used at the baseline study visit (SHIP-0) and the repeat year 5 study visit (SHIP-1) produced comparable SNP associations (Supplementary Material, Table S5).

IGF-I and IGFBP-3 concentrations were associated with height, weight and body circumferences (Supplementary Material, Table S6). The significant associations reported in Table 2 between SNPs and IGF-I or IGFBP-3 concentrations persisted after adjustment for these anthropometric variables (Supplementary Material, Table S7).

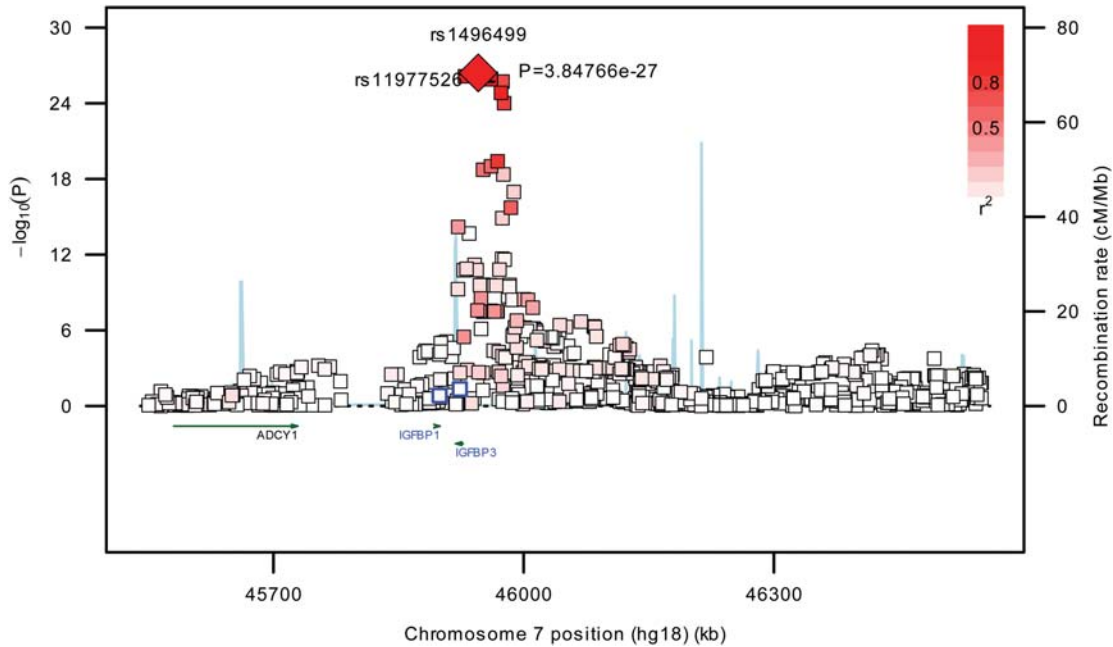


Figure 5. Regional plot for association of the *IGFBP3* region on chromosome 7 with IGFBP-3-adjusted IGF-I concentration. The diamond indicates rs1496499, the SNP with the lowest P for association with IGF-I concentration after adjustment for age, sex and IGFBP-3 concentration. Also shown is rs11977526, the SNP most strongly associated with IGFBP-3 levels (Fig. 1). The left y -axis indicates P for association with IGFBP-3-adjusted IGF-I concentration, and the right y -axis indicates recombination rate. The correlation between rs1496499 and rs11977526 was $r^2 = 0.72$.

We lacked directly genotyped information for four of the SNPs that were associated with circulating IGF-I or IGFBP-3 concentrations (rs11977526, rs1065656, rs7780564 and rs1245541, see Table 2 and Supplementary Material, Table S2). For each of these four SNPs, we identified the closest available SNP that was directly genotyped to serve as a proxy. The relationships with circulating IGF-I and IGFBP-3 concentrations were similar for the imputed-only SNPs (Table 2) and for the directly genotyped proxy SNPs (Supplementary Material, Table S8).

DISCUSSION

Both adult GH deficiency, which is characterized by low serum IGF-I values, and acromegaly, accompanied by IGF-I oversecretion, have been related to excess mortality, increased cardiovascular disease risk and impaired quality of life and functional abilities (25–28). Moreover, it has also been recognized that in adult populations without frank IGF system abnormalities, circulating IGF-I and IGFBP-3 concentrations independently predict risk of mortality and morbidity. Several large population-based cohorts revealed relationships of low circulating IGF-I concentrations, and both high and low IGFBP-3 concentrations, with higher mortality and greater incidence of cardiovascular conditions including ischemic heart disease and congestive heart failure (8–13,29). Furthermore, studies have suggested that high concentrations of IGF-I may be a risk factor for the development of cancers, including prostate and breast cancers (6,7). Thus, IGF-I and IGFBP-3 appear to mediate several clinical

conditions that are associated with substantial mortality, medical costs and loss of quality of life in middle-aged to older adults.

We confirmed an association of circulating IGFBP-3 levels with the *IGFBP3* gene region on chromosome 7p12.3. The most highly significant SNP, rs11977526, is in LD with a known region in *IGFBP3* (21–23). Another chromosome 7p12.3 SNP that was associated with IGFBP-3 concentrations, rs700752, is in a coding region called LOC730338;FLJ43321 but is not near any known gene and has no known function. rs700752 appeared to represent a second locus on 7p12.3 that is independent of the known *IGFBP3* variant. We had data on the known *IGFBP3* SNP in only one of the four study cohorts, which is a limitation. A third SNP associated with IGFBP-3 concentration, rs4234798, is located within *SORCS2*, a recently identified neuropeptide receptor gene. While little is known about *SORCS2* in humans, a related gene, *SORCS1*, has been identified as a type 2 diabetes locus (30,31). A fourth SNP, rs1065656, is ~ 1 kb away from the *IGFALS* gene, thus confirming the previously reported association of *IGFALS* with IGFBP-3 concentrations (24). The IGF acid-labile subunit (ALS) is a liver-derived glycoprotein that regulates IGF-I bioavailability by forming a ternary complex with IGF-I and IGFBP-3 in the circulation, thereby extending the circulating half-life of IGF-I and reducing its interaction with its receptors. Patients with *IGFALS* mutations are deficient in circulating IGF system proteins, but somewhat surprisingly have relatively modest decreases in linear growth (32,33).

We report two genome-wide significant SNP associations with circulating IGF-I concentrations. rs700752 on chromosome 7p12.3 was associated with both circulating IGF-I and

IGFBP-3 concentrations. The significant association of rs700752 with IGF-I concentrations was attenuated when analyses were conditioned on IGFBP-3 concentrations. In contrast, SNPs in the *IGFBP3* gene on chromosome 7 were associated with IGF-I concentrations only after adjustment for IGFBP-3 concentrations. These findings raise several intriguing questions. Do these chromosome 7 SNPs associate with IGF-I concentrations because of their effects on circulating IGFBP-3? Do these findings suggest the presence of a genomic region that might influence the affinity of binding between IGF-I and IGFBP-3? Unfortunately, measurement of free bioavailable IGF-I or IGF-I bioactivity has not been performed in our cohorts, which might have helped elucidate the binding mechanisms (34). Further research is needed to understand the direct or indirect mechanisms that link these SNPs with circulating IGF-I concentrations.

Several other SNPs had marginal associations with circulating IGF-I concentrations but did not meet levels of genome-wide significance. For example, rs1245541 is located in a region of chromosome 10 that has been associated with late-onset Alzheimer's disease (35). IGF-I may have neuroprotective effects and mediate clearance of beta-amyloid (36,37), suggesting a need to further investigate whether IGF-I may, in part, mediate the association of this chromosome 10 region with Alzheimer's disease. Another SNP associated with IGF-I concentrations, rs2153960, is located within *FOXO3* which regulates the expression of IGFBP-1 (38). Forkhead box O (FOXO) transcription factors induce cell growth arrest and apoptosis, which can be prevented by FOXO phosphorylation by the survival kinase AKT in response to growth factors such as IGF-I (39). In the absence of growth factors, FOXOs reside in the nucleus and up-regulate genes that inhibit the cell cycle, promote apoptosis and decrease oxidative stress (40). Prior human studies link variation in *FOXO3* with longevity (41). Therefore, the association of rs2153960 with IGF-I concentration is plausible and intriguing, even if it did not achieve pre-specified levels of genome-wide statistical significance.

The present study is the first, to our knowledge, that has used a GWA study approach in identifying genetic loci associated with circulating IGF-I and IGFBP-3 concentrations. Studies have found that candidate genes of the GH/IGF-I system such as *IGF1* (42,43), *IGF1R* (44), *IGFALS* (24), *GHI* (45) and *GHRHR* (46) may be associated with circulating IGF-I and IGFBP-3 concentrations and with related phenotypes such as breast cancer and colorectal cancer (Supplementary Material, Table S9). While the findings from our GWA study confirmed several likely IGF gene candidates (*IGFBP3*, *IGFALS*), they also suggest the existence of other important genes that regulate circulating IGF-I and IGFBP-3 concentrations.

Our study phenotypes included the main mediator of the GH/IGF axis, IGF-I, as well as the most abundant IGFBP, IGFBP-3. Study limitations include lack of phenotypic data on other components of this complex endocrine pathway, including circulating concentrations of GH, IGF-II, ALS and additional members of the IGFBP family. The four cohorts that were studied used different assay methodologies to measure IGF-I and IGFBP-3 concentrations, albeit that all were based on a similar analytical principle. Different assay

methodologies produce different estimates of circulating IGF-I concentrations, which represent an important problem in clinical practice (47). This design feature precluded us from combining effect estimates across the cohorts (within-cohort effect estimates appear in Supplementary Material, Table S2). We therefore accounted for variation across cohorts in assay methodologies through a z-score-based data analysis approach, as well as sensitivity analyses that showed consistent results when alternative assay methods were used at two different visits in the SHIP study. Another limitation is the use of imputed rather than directly genotyped information for some SNPs which reduced the effective sample size (48), albeit that in most cases the ratio of the empirically observed variance to the expected binomial variance was well above 0.8, indicating good imputation quality (Supplementary Material, Table S2).

In summary, we provide additional evidence for published associations between circulating IGFBP-3 concentrations and the *IGFBP3* (chromosome 7) and *IGFALS* (chromosome 16) gene regions, and identified a novel chromosome 4 region that was associated with IGFBP-3 concentrations. We also found associations of both IGFBP-3 concentrations and IGF-I concentrations with a second chromosome 7 SNP (rs700752) that was apparently independent of the SNPs near *IGFBP3*. In combination, these four loci together explained 6.5% of the inter-individual variation in circulating IGFBP-3 concentrations. IGF-I concentrations were associated with SNPs near *IGFBP3*, but only after adjustment for IGFBP-3 concentrations, suggesting that *IGFBP3* polymorphisms may influence the amount of free circulating IGF-I. The findings have implications for understanding the diverse influences that modulate the IGF system in adults.

MATERIALS AND METHODS

Study population

Details of study recruitment, data collection and genotyping appear in Supplementary Materials. We studied men and women enrolled in four community-based epidemiological cohort studies in the USA and Germany: CHS, FHS, KORA and SHIP. The present study was limited to individuals who were of European or Caucasian origin as ascertained by self-report. Individuals with diabetes mellitus or reduced renal function (estimated glomerular filtration rate <50 ml/min/1.73 m²) were excluded. All participants provided informed consent and human subjects' research review was obtained from each participating cohort.

IGF-I and IGFBP-3 measurements

IGF-I and IGFBP-3 measurements were performed by each cohort study group using stored blood specimens (Supplementary Material, Table S1). In CHS, assays were performed using enzyme-linked immunosorbent assay (ELISA) methods in fasting plasma using reagents from Diagnostic Systems Laboratory. In FHS, assays were performed on fasting serum using ELISA methods with R&D Systems reagents. In KORA, assays were performed on fasting plasma using automated chemiluminescent immunometric assays (Siemens). In

SHIP, IGF-I and IGFBP-3 measurements were made on non-fasting serum from two study visits. At the baseline SHIP visit (SHIP-0), assays were performed using automated two-site chemiluminescence immunoassays (Nichols Advantage). Repeated non-fasting serum measurements were made at a follow-up visit after 5 years (SHIP-1), using Siemens automated chemiluminescent immunometric assays. For the primary analyses, we used data from the SHIP-0 visit, and we also repeated the analyses using data from the SHIP-1 visit; within-person correlations of the repeated measurements were moderate to high (for IGF-I, $r^2 = 0.71$, for IGFBP-3, $r^2 = 0.63$). Assay CVs were below 10% in each cohort.

Genotyping and statistical analyses

Genotyping was performed by each cohort using methods described in Table 1. SNP data were imputed using the HapMap CEU population to harmonize data across the genotyping platforms used by the four cohorts (~2.5 million SNPs). Data analyses used an additive model relating SNPs with IGF-I or IGFBP-3 concentration after adjustment for age and sex. *P*-values from within-cohort results and meta-analysis results were corrected for genomic control (λ -values were 1.03 for analyses of IGF-I concentrations, 1.02 for analyses of IGFBP-3 concentrations and 1.02 for analyses of IGFBP-3-adjusted IGF-I concentrations). Meta-analysis was used to combine study results using a *z*-score-based approach, with weighting by effective sample size which was calculated as the total sample size multiplied by the imputation quality ratio for the SNP. The *z*-score-based meta-analysis approach was chosen to account for the non-uniformity in IGF-I and IGFBP-3 assay methods across study cohorts. The total number of individuals in the study was 10 280 for analyses of IGF-I concentrations and 10 018 for analyses of IGFBP-3 concentrations. Individual SNPs were excluded if they had an effective sample size below 8000 in the meta-analysis. A significance threshold of $P < 5 \times 10^{-8}$ was used as the criterion for genome-wide significance. We estimated the percentage of between-individual variation in circulating IGFBP-3 concentrations that was explained by the genome-wide significant SNPs. This was derived in a linear regression model that contained all genome-wide significant loci, age and sex as independent variables; both cohort-specific and weighted average estimates of r^2 were estimated. Because most circulating IGF-I is bound to IGFBP-3, the amount of free IGF-I in circulating blood is strongly influenced by the blood IGFBP-3 concentration. Therefore, we also conducted analyses to identify SNPs associated with IGF-I concentrations after adjustment for IGFBP-3 concentrations.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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REFERENCES

- Le Roith, D. (1997) Seminars in medicine of the Beth Israel Deaconess Medical Center. Insulin-like growth factors. *N. Engl. J. Med.*, **336**, 633–640.
- Franklin, S.L., Ferry, R.J. Jr and Cohen, P. (2003) Rapid insulin-like growth factor (IGF)-independent effects of IGF binding protein-3 on endothelial cell survival. *J. Clin. Endocrinol. Metab.*, **88**, 900–907.
- Rajah, R., Lee, K.W. and Cohen, P. (2002) Insulin-like growth factor binding protein-3 mediates tumor necrosis factor- α -induced apoptosis: role of Bcl-2 phosphorylation. *Cell Growth Differ.*, **13**, 163–171.
- Muzumdar, R.H., Ma, X., Fishman, S., Yang, X., Atzmon, G., Vuguin, P., Einstein, F.H., Hwang, D., Cohen, P. and Barzilai, N. (2006) Central and opposing effects of IGF-I and IGF-binding protein-3 on systemic insulin action. *Diabetes*, **55**, 2788–2796.
- Kenyon, C.J. (2010) The genetics of ageing. *Nature*, **464**, 504–512.
- Samani, A.A., Yakar, S., LeRoith, D. and Brodt, P. (2007) The role of the IGF system in cancer growth and metastasis: overview and recent insights. *Endocr. Rev.*, **28**, 20–47.
- Renahan, A.G., Zwaahlen, M., Minder, C., O'Dwyer, S.T., Shalet, S.M. and Egger, M. (2004) Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. *Lancet*, **363**, 1346–1353.
- Juul, A., Scheike, T., Davidsen, M., Gyllenborg, J. and Jorgensen, T. (2002) Low serum insulin-like growth factor I is associated with increased risk of ischemic heart disease: a population-based case-control study. *Circulation*, **106**, 939–944.
- Vasan, R.S., Sullivan, L.M., D'Agostino, R.B., Roubenoff, R., Harris, T., Sawyer, D.B., Levy, D. and Wilson, P.W. (2003) Serum insulin-like growth factor I and risk for heart failure in elderly individuals without a previous myocardial infarction: the Framingham Heart Study. *Ann. Intern. Med.*, **139**, 642–648.
- Saydah, S., Graubard, B., Ballard-Barbash, R. and Berrigan, D. (2007) Insulin-like growth factors and subsequent risk of mortality in the United States. *Am. J. Epidemiol.*, **166**, 518–526.
- Laughlin, G.A., Barrett-Connor, E., Criqui, M.H. and Kritiz-Silverstein, D. (2004) The prospective association of serum insulin-like growth factor I (IGF-I) and IGF-binding protein-1 levels with all cause and cardiovascular disease mortality in older adults: the Rancho Bernardo Study. *J. Clin. Endocrinol. Metab.*, **89**, 114–120.
- Roubenoff, R., Parise, H., Payette, H.A., Abad, L.W., D'Agostino, R., Jacques, P.F., Wilson, P.W., Dinarello, C.A. and Harris, T.B. (2003) Cytokines, insulin-like growth factor 1, sarcopenia, and mortality in very old community-dwelling men and women: the Framingham Heart Study. *Am. J. Med.*, **115**, 429–435.
- Friedrich, N., Haring, R., Nauck, M., Ludemann, J., Roszkopf, D., Spilcke-Liss, E., Felix, S.B., Dorr, M., Brabant, G., Volzke, H. *et al.* (2009) Mortality and serum insulin-like growth factor (IGF)-I and IGF binding protein 3 concentrations. *J. Clin. Endocrinol. Metab.*, **94**, 1732–1739.
- Heald, A.H., Cruickshank, J.K., Riste, L.K., Cade, J.E., Anderson, S., Greenhalgh, A., Sampayo, J., Taylor, W., Fraser, W., White, A. *et al.* (2001) Close relation of fasting insulin-like growth factor binding protein-1 (IGFBP-1) with glucose tolerance and cardiovascular risk in two populations. *Diabetologia*, **44**, 333–339.
- Creutzberg, E.C. and Casaburi, R. (2003) Endocrinological disturbances in chronic obstructive pulmonary disease. *Eur. Respir. J. Suppl.*, **46**, 76s–80s.
- Vogiatzis, I., Stratakos, G., Simoes, D.C., Terzis, G., Georgiadou, O., Roussos, C. and Zakyntinos, S. (2007) Effects of rehabilitative exercise on peripheral muscle TNF α , IL-6, IGF-I and MyoD expression in patients with COPD. *Thorax*, **62**, 950–956.
- Feldt-Rasmussen, B. and El Nahas, M. (2009) Potential role of growth factors with particular focus on growth hormone and insulin-like growth factor-1 in the management of chronic kidney disease. *Semin. Nephrol.*, **29**, 50–58.
- Harrela, M., Koistinen, H., Kaprio, J., Lehtovirta, M., Tuomilehto, J., Eriksson, J., Toivanen, L., Koskenvuo, M., Leinonen, P., Koistinen, R. *et al.* (1996) Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. *J. Clin. Invest.*, **98**, 2612–2615.
- Souren, N.Y., Paulussen, A.D., Loos, R.J., Gielen, M., Beunen, G., Fagard, R., Derom, C., Vlietinck, R. and Zeegers, M.P. (2007) Anthropometry, carbohydrate and lipid metabolism in the East Flanders Prospective Twin Survey: heritabilities. *Diabetologia*, **50**, 2107–2116.
- Hong, Y., Pedersen, N.L., Brisman, K., Hall, K. and de Faire, U. (1996) Quantitative genetic analyses of insulin-like growth factor I (IGF-I), IGF-binding protein-1, and insulin levels in middle-aged and elderly twins. *J. Clin. Endocrinol. Metab.*, **81**, 1791–1797.
- Deal, C., Ma, J., Wilkin, F., Paquette, J., Rozen, F., Ge, B., Hudson, T., Stampfer, M. and Pollak, M. (2001) Novel promoter polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. *J. Clin. Endocrinol. Metab.*, **86**, 1274–1280.
- Canzian, F., McKay, J.D., Cleveland, R.J., Dossus, L., Biessy, C., Rinaldi, S., Landi, S., Boillot, C., Monnier, S., Chajes, V. *et al.* (2006) 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*, **94**, 299–307.
- Schumacher, F.R., Cheng, I., Freedman, M.L., Mucci, L., Allen, N.E., Pollak, M.N., Hayes, R.B., Stram, D.O., Canzian, F., Henderson, B.E. *et al.* (2010) A comprehensive analysis of common IGF1, IGFBP1 and IGFBP3 genetic variation with prospective IGF-I and IGFBP-3 blood levels and prostate cancer risk among Caucasians. *Hum. Mol. Genet.*, **19**, 3089–3101.
- Gu, F., Schumacher, F.R., Canzian, F., Allen, N.E., Albanes, D., Berg, C.D., Berndt, S.I., Boeing, H., Bueno-de-Mesquita, H.B., Buring, J.E. *et al.* (2010) 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. *Cancer Epidemiol. Biomarkers Prev.* (epub September 1, 2010).
- Colao, A., Ferone, D., Marzullo, P. and Lombardi, G. (2004) Systemic complications of acromegaly: epidemiology, pathogenesis, and management. *Endocr. Rev.*, **25**, 102–152.
- Mathioudakis, N. and Salvatori, R. (2008) Adult-onset growth hormone deficiency: causes, complications and treatment options. *Curr. Opin. Endocrinol. Diabetes Obes.*, **15**, 352–358.
- Moock, J., Albrecht, C., Friedrich, N., Volzke, H., Nauck, M., Koltowska-Haggstrom, M., Kohlmann, T. and Wallaschofski, H. (2009) Health-related quality of life and IGF-1 in GH-deficient adult patients on GH replacement therapy: analysis of the German KIMS data and the Study of Health in Pomerania. *Eur. J. Endocrinol.*, **160**, 17–24.
- Clayton, R.N. (2003) Cardiovascular function in acromegaly. *Endocr. Rev.*, **24**, 272–277.
- Kaplan, R.C., McGinn, A.P., Pollak, M.N., Kuller, L.H., Strickler, H.D., Rohan, T.E., Cappola, A.R., Xue, X. and Psaty, B.M. (2007) Association of total insulin-like growth factor-I, insulin-like growth factor binding protein-1 (IGFBP-1), and IGFBP-3 levels with incident coronary events and ischemic stroke. *J. Clin. Endocrinol. Metab.*, **92**, 1319–1325.
- Rezgaoui, M., Hermey, G., Riedel, I.B., Hampe, W., Schaller, H.C. and Hermans-Borgmeyer, I. (2001) Identification of SorCS2, a novel member of the VPS10 domain containing receptor family, prominently expressed in the developing mouse brain. *Mech. Dev.*, **100**, 335–338.
- Goodarzi, M.O., Lehman, D.M., Taylor, K.D., Guo, X., Cui, J., Quinones, M.J., Clee, S.M., Yandell, B.S., Blangero, J., Hsueh, W.A. *et al.* (2007) SORCS1: a novel human type 2 diabetes susceptibility gene suggested by the mouse. *Diabetes*, **56**, 1922–1929.
- Domene, H.M., Bengolea, S.V., Martinez, A.S., Ropelato, M.G., Pennisi, P., Scaglia, P., Heinrich, J.J. and Jasper, H.G. (2004) Deficiency of the circulating insulin-like growth factor system associated with inactivation of the acid-labile subunit gene. *N. Engl. J. Med.*, **350**, 570–577.
- Domene, H.M., Hwa, V., Argente, J., Wit, J.M., Camacho-Hubner, C., Jasper, H.G., Pozo, J., van Duyvenvoorde, H.A., Yakar, S., Fofanova-Gambetti, O.V. *et al.* (2009) Human acid-labile subunit deficiency: clinical, endocrine and metabolic consequences. *Horm. Res.*, **72**, 129–141.
- Frystyk, J. (2007) Utility of free igf-I measurements. *Pituitary*, **10**, 181–187.

35. Myers, A., Wavrant De-Vrieze, F., Holmans, P., Hamshere, M., Crook, R., Compton, D., Marshall, H., Meyer, D., Shears, S., Booth, J. *et al.* (2002) Full genome screen for Alzheimer disease: stage II analysis. *Am. J. Med. Genet.*, **114**, 235–244.
36. Carro, E., Trejo, J.L., Gomez-Isla, T., LeRoith, D. and Torres-Aleman, I. (2002) Serum insulin-like growth factor I regulates brain amyloid-beta levels. *Nat. Med.*, **8**, 1390–1397.
37. Dore, S., Kar, S. and Quirion, R. (1997) Insulin-like growth factor I protects and rescues hippocampal neurons against beta-amyloid- and human amylin-induced toxicity. *Proc. Natl Acad. Sci. USA*, **94**, 4772–4777.
38. Gan, L., Han, Y., Bastianetto, S., Dumont, Y., Unterman, T.G. and Quirion, R. (2005) FoxO-dependent and -independent mechanisms mediate SirT1 effects on IGFBP-1 gene expression. *Biochem. Biophys. Res. Commun.*, **337**, 1092–1096.
39. Huang, H. and Tindall, D.J. (2007) Dynamic FoxO transcription factors. *J. Cell Sci.*, **120**, 2479–2487.
40. Paik, J.H., Kollipara, R., Chu, G., Ji, H., Xiao, Y., Ding, Z., Miao, L., Tothova, Z., Horner, J.W., Carrasco, D.R. *et al.* (2007) FoxOs are lineage-restricted redundant tumor suppressors and regulate endothelial cell homeostasis. *Cell*, **128**, 309–323.
41. Willcox, B.J., Donlon, T.A., He, Q., Chen, R., Grove, J.S., Yano, K., Masaki, K.H., Willcox, D.C., Rodriguez, B. and Curb, J.D. (2008) FOXO3A genotype is strongly associated with human longevity. *Proc. Natl Acad. Sci. USA*, **105**, 13987–13992.
42. Rietveld, I., Janssen, J.A., Hofman, A., Pols, H.A., van Duijn, C.M. and Lamberts, S.W. (2003) A polymorphism in the IGF-I gene influences the age-related decline in circulating total IGF-I levels. *Eur. J. Endocrinol.*, **148**, 171–175.
43. Missmer, S.A., Haiman, C.A., Hunter, D.J., Willett, W.C., Colditz, G.A., Speizer, F.E., Pollak, M.N. and Hankinson, S.E. (2002) A sequence repeat in the insulin-like growth factor-1 gene and risk of breast cancer. *Int. J. Cancer*, **100**, 332–336.
44. Suh, Y., Atzmon, G., Cho, M.O., Hwang, D., Liu, B., Leahy, D.J., Barzilai, N. and Cohen, P. (2008) Functionally significant insulin-like growth factor I receptor mutations in centenarians. *Proc. Natl Acad. Sci. USA*, **105**, 3438–3442.
45. Le Marchand, L., Donlon, T., Seifried, A., Kaaks, R., Rinaldi, S. and Wilkens, L.R. (2002) Association of a common polymorphism in the human GH1 gene with colorectal neoplasia. *J. Natl Cancer Inst.*, **94**, 454–460.
46. Canzian, F., McKay, J.D., Cleveland, R.J., Dossus, L., Biessy, C., Boillot, C., Rinaldi, S., Llewellyn, M., Chajes, V., Clavel-Chapelon, F. *et al.* (2005) Genetic variation in the growth hormone synthesis pathway in relation to circulating insulin-like growth factor-I, insulin-like growth factor binding protein-3, and breast cancer risk: results from the European prospective investigation into cancer and nutrition study. *Cancer Epidemiol. Biomarkers Prev.*, **14**, 2316–2325.
47. Frystyk, J., Freda, P. and Clemmons, D.R. (2010) The current status of IGF-I assays—a 2009 update. *Growth Horm. IGF Res.*, **20**, 8–18.
48. Biernacka, J.M., Tang, R., Li, J., McDonnell, S.K., Rabe, K.G., Sinnwell, J.P., Rider, D.N., de Andrade, M., Goode, E.L. and Fridley, B.L. (2009) Assessment of genotype imputation methods. *BMC Proc.*, **3**, S5–S6.