Novel Promoter Polymorphism in Insulin-Like Growth Factor-Binding Protein-3: Correlation with Serum Levels and Interaction with Known Regulators*

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ABSTRACT

Insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) is a major determinant of circulating levels of the IGFs and is clinically useful for the evaluation of GH deficiency and for predicting the response to GH treatment. Recent studies provide evidence that the circulating level of IGFBP-3 is inversely related to the risk of several common cancers, and that antiproliferative agents such as antiestrogens and retinoids act in part by up-regulating IGFBP-3 gene (*IGFBP3*) expression. Although approximately 50% of the substantial interindividual variability in circulating IGFBP-3 levels is known to have a genetic basis, the specific loci involved are unknown.

Direct sequencing of genomic DNA specimens from a multiethnic population identified several single nucleotide polymorphisms in the promoter region of *IGFBP3*. For the most common single nucleotide polymorphism (nucleotide −202) found to be in Hardy-Weinberg equilibrium, genotype was highly correlated to circulating level of IGFBP-3 in 478 men from the Physicians' Health Study. *In vitro*, we documented significantly higher promoter activity of the A allele at the −202 locus compared with the C allele, consistent with the relationship observed between genotype and circulating IGFBP-3 (AA > AC > CC).

A positive correlation was observed between circulating retinol levels and circulating IGFBP-3 levels; subset analysis by genotype showed that this relationship was only present among individuals carrying an A allele at −202 (AA > AC > CC). Tall individuals or individuals with a body mass index of 27 or greater had levels of circulating IGFBP-3 that were significantly higher when they possessed at least one A allele (AA > AC > CC).

The *IGFBP3* promoter region deserves investigation as a locus where polymorphic variation occurs frequently and may influence GH responsiveness, somatic growth, and possibly cancer risk.

THE INSULIN-LIKE GROWTH factors (IGF-I and IGF-II), their receptors (type 1 and type 2), and their binding proteins (IGFBPs) play key roles in regulating cell proliferation and apoptosis (1) and have been clearly associated with neoplasia (2). IGFBP-3 has several physiological roles. Not only is it the major carrying protein for IGF-I and IGF-II in the circulation, but it also acts as a modulator of IGF bioactivity and as a direct growth inhibitor in the extravascular tissue compartment, where it is expressed in a highly regulated manner (3). Circulating IGF-I and IGFBP-3 levels vary greatly between normal individuals, but until recently less attention was given to the study of interindividual variability within the broad normal range than to the extremes defined by GH deficiency (associated with low levels) and acromegaly (associated with high levels). Interest in the variability of IGF-I and IGFBP-3 within the normal range has increased (4–6) after several reports that circulating IGF-I levels are positively correlated with the risk of several common cancers and that high IGFBP-3 levels tend to attenuate risk (7–12). Furthermore, circulating IGF-I and IGFBP-3 levels and their ratio are known to be related to somatic growth (13, 14), and there is evidence that both height and leg length are related to cancer risk (15–17).

The gene for IGFBP-3 (*IGFBP3*) is highly conserved among species and is present as a single copy on chromosome 7p14-p12. GH and insulin are two of the hormones important in *IGFBP3* up-regulation (18), in addition to agents that induce growth inhibition/apoptosis such as p53 (19), retinoic acid (20, 21), vitamin D (22), antiestrogens such as tamoxifen (23), antiandrogens (24), transforming growth factor-β (21, 25), and tumor necrosis factor-α (26). There is evidence that this up-regulation contributes to the antiproliferative actions of some of the drugs used in chemoprevention (2, 20, 21, 23, 27). In addition, the growth-promoting action of estrogens and ligands for the epidermal growth factor receptor have been shown to down-regulate *IGFBP3* expression (18).

Twin studies (28) have shown that about half of the intraindividual variability in circulating IGFBP-3 levels is genetically determined, but specific loci involved have not been described. We therefore undertook studies to determine

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whether variability in IGFBP-3 levels might be related to polymorphic variants of the promoter region of the gene.

Materials and Methods

Subjects and samples

Approximately 1.5 kb of the promoter region of *IGFBP3* (see below) was sequenced for each of 32 samples of genomic DNA from a multiethnic Montréal population to identify possible polymorphisms. We then genotyped these loci by direct sequencing in 40 leukocyte DNA specimens from subjects in the Physicians' Health Study (29). IGF-I, IGF-II, and IGFBP-3 levels had previously been measured in corresponding plasma samples, using enzyme-linked immunosorbent assay methods (9). This pilot work suggested that a single nucleotide polymorphism at position −202 (nucleotides relative to the CAP site) was related to the circulating IGFBP-3 concentration, because mean IGFBP-3 levels were higher in subjects with the AA genotype than in those with the CC genotype ($P < 0.05$, by unpaired t test). To explore this possibility in more detail, we developed a restriction fragment length polymorphism (RFLP) assay and genotyped 524 subjects at the −202 locus (colon cancer cases matched to controls 1:2) from the Physicians' Health Study. For 478 of these, we had previously (9) measured IGF-I, IGF-II, and IGFBP-3 plasma levels, height, and weight, and for 283 of these subjects, plasma retinol levels were also determined. All subjects gave informed consent for samples they provided to be used for scientific research.

IGFBP3 promoter polymorphism analysis

The following primer pairs were used to sequence *IGFBP3* from −1500 to +19 relative to the CAP site (*underlined* portion corresponds to the M13 tail) in the initial screening of an ethnically mixed Montréal population: BP3Prom1F, 5′-GTTTTCCCAGTCACGACGTACAAGAGGGAACGAAATTGATC-3′; BP3Prom1R, 5′-TTGTCTGCGCACCCCCTCCTT-3′ (Tm = 55 C); BP3Prom2F, 5′-GTTTTCCCAGTCACGACAGGAAAGTCTCCTCCCGCGGA-3′; BP3Prom2R, 5′-GCTCCTTAAGGCAGGGCTTTTC-3′ (Tm = 54 C); BP3Prom3F, 5′-GTTTTCCCAGTCACGACGTACACGTTTCAGCAGTGCCCAGTTTA-3′; BP3Prom3R, 5′-TTCCGCTCTCGGGGTGAGGTCT-3′ (Tm = 52 C); BP3Prom4F, 5′-GTTTTCCCAGTCACGACCCACGAGGTACACACGAATG-3′; and BP3Prom4R, 5′-AGCCGCAGTGCTCGCATCTGG-3′ (Tm = 64 C).

Initial PCR conditions consisted of 25 ng genomic DNA, 1 U AmpliTaq Gold DNA Polymerase (Perkin-Elmer Corp., Mississaugua, Canada), 1 × PCR Gold Buffer (Perkin-Elmer Corp.), 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of each deoxy-NTP, and 2% dimethylsulfoxide in a total volume of 50 μL. After denaturation at 96 C for 10 min, 35 cycles were run with the following cycling parameters: 96 C for 30 s, Tm (see above) for 30 s, and 72 C for 1 min. The PCR reaction was purified on magnetic beads and then directly sequenced.

RFLP genotyping of the −202 single nucleotide polymorphism

A 50- to 250-ng aliquot of genomic DNA was mixed with PCR buffer, supplemented by 1.6 μmol/L of primers, 1 mmol/L $MgCl_2$, 0.1 mmol/L of each deoxy-NTP, 2% dimethyl sulfoxide 2 U *Taq* DNA polymerase (Life Technologies, Inc., Burlington, Canada). Primer sense and antisense were 5′-CCA CGA GGT ACA CAC GAA TG-3′ and 5′-AGC CGC AGT GCT CGC ATC TGG-3′, respectively. The cycling parameters consisted of an initial incubation of 10 min at 94 C, followed by 35 cycles of 30 s at 96 C, 30 s at 64 C, and 1 min at 72 C. The reaction was terminated after a final extension of 5 min at 72 C. Twenty microliters of PCR product were digested with 5 U *Alw*21I (MBI Fermentas, Flamborough, Canada) from between 3–14 h at 37 C. Digestion products were visualized on a 2% agarose gel stained with ethidium bromide. Due to the presence of three *Alw*21I sites in the PCR product, one of which is destroyed when there is a C in position −202, band sizes were 242 and 162 bp (A allele), and 288 and 162 bp (C allele).

Promoter activity assay

PCR products for human *IGFBP3* promoter (bp −441 to +91) (30) were obtained from samples with A or C at bp −202. PCR conditions were the same as described above, except for the addition of linkers with restriction sites in 28-mer primers. The sense primer containing a *Pst*I site was 5′-AAC TGC AGC CAC GAG GTA CAC ACG AAT G-3′, and the antisense primer containing an *Xba*I was 5′-GCT CTA GAC GCA GGG ATG GGG CGA CAG T-3′. To generate *IGFBP3* reporter gene constructs for transient transfections, we cloned the two different 548-bp PCR products into the *Pst*I/*Xba*I sites of pCAT-Basic plasmid (Promega Corp., Madison, WI). These constructs were termed pCAT BP3A and pCAT BP3C. As controls, we used the promoterless pCAT-Basic plasmid and the pCAT-control plasmid containing simian virus 40 promoter and enhancer. The authenticity and directionality of all of the constructs were verified by restriction enzyme analysis and were confirmed by sequencing both sense and antisense strands. *IGFBP3*-expressing human liver cancer SK-Hep-1 cells and MCF-7 breast cancer cells obtained from American Type Culture Collection (Manassas, VA) were used for transient transfections. Briefly, 1.5×10^5 MCF-7 cells or 3×10^5 SK-Hep-1 cells in 35-cm^2 multiwell dishes in serum-free medium (Opti-MEM, Life Technologies, Inc.) were cotransfected with 2 μg of each chloramphenicol acetyltransferase (CAT) construct and with 0.7 μg pSVβ (a plasmid encoding β-galactosidase, Promega Corp.) using a cationic liposome formulation (lipofectin, Life Technologies, Inc.). The CAT and β-galactosidase assays were then performed 48 h later as previously detailed (31).

Statistical analyses

We assessed the genotype distributions for significant departure from the Hardy-Weinberg equilibrium using the χ^2 test. A general linear regression model was employed to estimate the percent variation in IGFBP-3 that can be explained by the −202 polymorphism alone or by the genotype and age. Analysis of covariance was used to compare the age-adjusted levels of IGFBP-3 according to the genotype or plasma retinol levels. Age-adjusted mean levels were also obtained according to the genotype and tertile of plasma retinol, quartile of height, or four groups of body mass index among case subjects and control subjects. Unconditional logistic regression was used to estimate the relative risks (RRs) and 95% confidence intervals (CIs) for association of the −202 polymorphism with the risk of colorectal cancer. The RRs were also calculated adjusting for age, smoking, IGF-I, body mass index (BMI), and alcohol intake. All P values are two-sided, and all analyses used the SAS program package (SAS Institute, Inc., Cary, NC).

Results

Five polymorphic loci in *IGFBP3* were detected (Table 1), and the genotype at one of these loci (−202) appeared to be significantly related to the age-adjusted circulating IGFBP-3 concentration. This locus is near elements believed to direct basal promoter activity of *IGFBP3* (18, 30) (Fig 1) and can be conveniently genotyped by an RFLP assay (Fig 2).

Polymorphic variation at the −202 locus was common in the study population (genotype distribution: AA = 120, AC = 247, CC = 157), and the genotype frequencies conformed to the Hardy-Weinberg equilibrium test ($\chi^2 = 0.98$; $P = 0.61$).

As shown in Fig. 3, mean IGFBP-3 levels were highest in the individuals with the AA genotype at the −202 *IGFBP3* locus and declined significantly in a stepwise manner in the

TABLE 1. Polymorphisms in the *IGFBP3* promoter region

Polymorphism	Nucleotide Position Relative to CAP Site	GenBank Position #M35878	Allele Frequency [no. (%)]
G→A	−795	1111	18/64 (28)
G→A	−667	1239	8/58 (14)
C→T	−396	1510	2/60 (3.3)
A→C	−202	1704	24/60 (40)
C→T	−185	1721	19/60 (32)

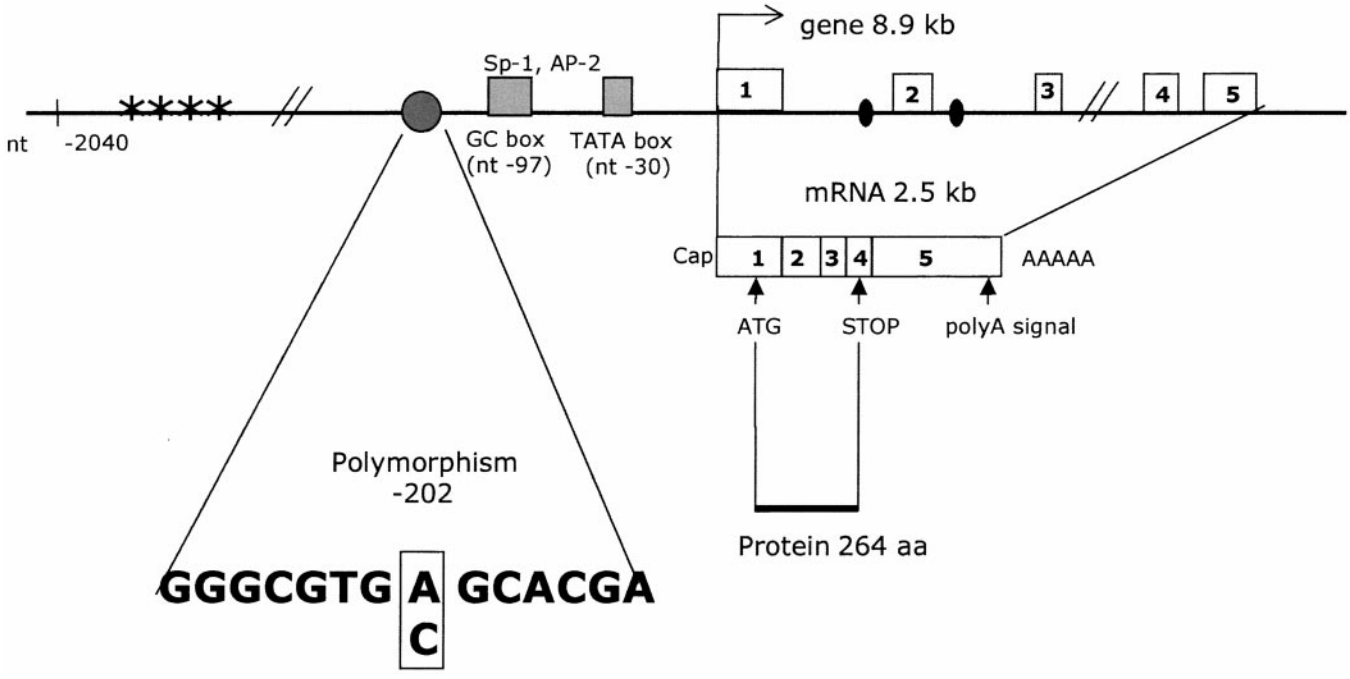

FIG. 1. Schematic diagram of the human IGFBP-3 gene. The location of the −202 polymorphism relative to the basal promoter (containing a GC box and TATA box) as described by Cubbage *et al.* (30) is shown. ****, Putative response elements for various hormone receptors and transcription factors that are located upstream (nucleotides −2000 to −200, relative to the CAP/transcription start site) in the rat gene (44) and in the bovine gene (45), many of which are conserved in the human. These include insulin, GH, retinoic acid, vitamin D, estrogen, thyroid hormone, glucocorticoids, tumor necrosis factor-α and -β, and epidermal growth factor. The *black ovals* indicate p53-binding sites in the human gene (19).

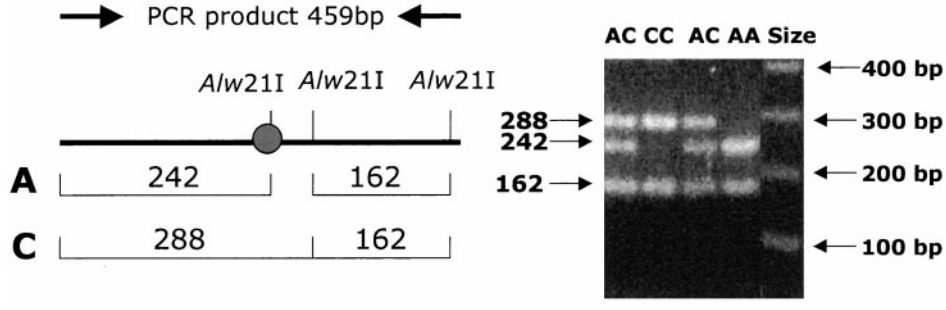

FIG. 2. PCR product of 459 bp (−441 to +19 relative to the cap site) for analysis of the −202 polymorphism. The schematic shows the expected fragments for the C or A allele upon digestion with the restriction enzyme *Alw*21I. A representative RFLP gel is shown to the *right* of the schematic, indicating the different genotypes in four individuals. Size markers are included in the *far right* lane.

presence of one or two copies of the C allele. The relationship between genotype and circulating serum IGFBP-3 level was apparent with or without adjustment for age. Using a general linear model, we estimated that the percent variation in IGFBP-3 that can be explained by the −202 polymorphism is 7.7% (r = 0.28); if both polymorphism and subject age are included, 22.3% of the interindividual variability in IGFBP-3 can be accounted for (r = 0.47). As anticipated, the −202 CC genotype was significantly associated with the total circulating total IGF (IGF-I + IGF-II) level in a dose-dependent fashion [AA *vs.* AC, 847 ± 15 and 792 ± 10 ng/mL, respectively ($P = 0.002$); AA *vs.* CC, 847 ± 15 and 759 ± 13 ng/mL, respectively ($P = 0.0001$)]. The −202 genotype was also predictive of IGF-I/IGFBP-3 molar ratios (AA *vs.* CC, 0.211 ± 0.005 and 0.248 ± 0.004, respectively; $P < 0.0001$) and IGF-II/IGFBP-3 molar ratios (AA *vs.* CC, 1.018 ± 0.008 and 1.044 ± 0.007, respectively; $P = 0.02$).

In view of the prior reports showing an inverse relation between circulating IGFBP-3 level and colorectal cancer risk (9, 12), we examined the possibility that colorectal cancer risk might be related to genotype at the −202 *IGFBP3* locus. There was no statistically significant relationship between genotype and RR of colorectal cancer, although there was a trend in this direction. Using the CC genotype as a reference (RR = 1.0), the RR associated with the AA genotype was 0.91 (95% CI = 0.56–1.49), and the RR associated with the AC genotype was 0.97 (95% CI = 0.64–1.46).

Expression of *IGFBP3 in vitro* is known to be up-regulated by retinoids (20, 21), but there have been no studies of circulating IGFBP-3 levels in relation to circulating retinol levels. Data for retinol levels, IGFBP-3 levels, and *IGFBP3* −202 genotype were available for 283 subjects. Figure 4 summarizes the relationships among these variables. Age-adjusted IGFBP-3 levels increased across tertiles of plasma retinol (trend analysis: $P < 0.005$; Spearman correlation coefficient r = 0.25; $P < 0.001$; Fig. 4A). When the age-adjusted mean levels of IGFBP-3 in each retinol tertile were analyzed after stratification by genotype at the −202 locus, lower levels of

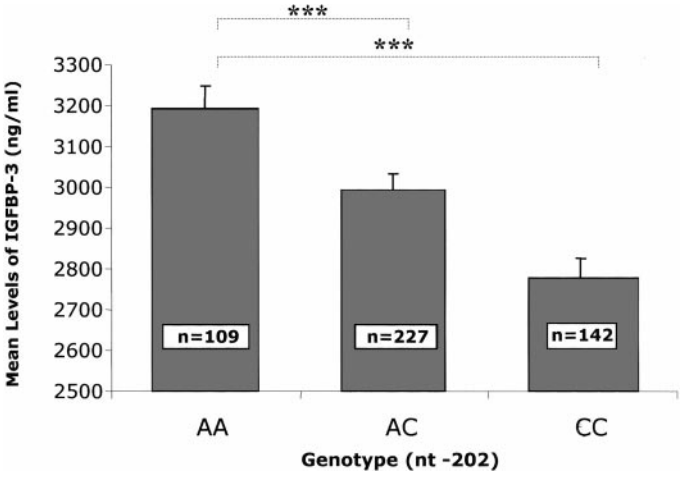

FIG. 3. Mean IGFBP-3 levels in all subjects according to genotype at nucleotide −202. The number of individuals analyzed (n) is indicated for each genotype (***, $P < 0.001$), and data are expressed as the mean ± SE.

IGFBP-3 were consistently observed with the CC genotype regardless of retinol levels (Fig. 4B). Importantly, the relationship of retinol level to IGFBP-3 level documented in Fig. 4A was strongly dependent on genotype at the *IGFBP3* −202 locus. Only the A allele, especially the AA genotype, was associated with progressively higher levels of IGFBP-3 with increasing retinol levels (overall IGFBP-3 level differed across retinol tertiles, $P = 0.001$; within AA genotype, retinol tertile 1 *vs.* 2, $P = 0.04$; tertile 1 *vs.* 3, $P = 0.0001$; within other genotypes, differences in IGFBP-3 levels across retinol tertiles were not significant).

The influence of the *IGFBP3* −202 polymorphism on circulating IGFBP-3 levels was also modified by BMI and height (Tables 2 and 3). We noted that the AA genotype (associated with higher IGFBP-3 levels) was overrepresented in shorter subjects (28% of subjects in the first height quartile subjects were AA, *vs.* 20% of subjects in the fourth height quartile; Table 3), whereas the CC genotype was more prevalent among taller individuals (31% of subjects in the fourth height quartile were CC, *vs.* 25% of subjects in the first height quartile; Table 3), but these trends were of only borderline significance ($P_{trend} = 0.05$ and 0.04, respectively). Overall, neither BMI nor height was related to the age-adjusted circulating IGFBP-3 level. However, both height and BMI influenced the relationship between the −202 *IGFBP3* genotype and the circulating IGFBP-3 level. The polymorphism was less significantly related to levels in shorter or leaner individuals than in taller and larger subjects (Tables 2 and 3).

The consistent finding that the CC genotype was associated with the lowest levels of IGFBP-3 and the AA genotype with the highest levels (with the AC genotype showing intermediate levels) regardless of the various ways our data were stratified prompted us to undertake preliminary functional studies of the *IGFBP3* promoter. Figure 5 shows the results of transient transfection experiments, in which two *IGFBP3* promoter constructs that differed only at the −202 locus were cotransfected with a β-galactosidase-containing plasmid (to monitor transfection efficiency). The activity of the reporter gene CAT in the transfectants containing the BP3-C construct (normalized to β-galactosidase activity) was approximately half that of CAT activity in transfectants containing the BP3-A construct. Similar results were obtained using SK-Hep-1 and MCF-7 cells as transfection targets, providing *in vitro* evidence that the −202 *IGFBP3* polymorphism influences promoter activity and, hence, expression of the gene.

Discussion

Circulating levels (and presumably tissue expression) of IGFBP-3 vary considerably between normal individuals. The gene encoding IGFBP-3 is highly expressed in liver, where the bulk of the circulating protein originates, and it is also expressed in a highly regulated fashion in the various tissues in which it influences cell renewal kinetics (1, 3, 18). A prior study provided evidence that about half of the interindividual heterogeneity in circulating IGFBP-3 levels was attributable to uncharacterized genetic factors (28).

Our main finding is that the novel −202 polymorphism in the promoter region of *IGFBP3* is related to the circulating IGFBP-3 level clinically and to the promoter activity of the gene *in vitro*. *In vitro* gene expression varied by approximately 50% between A- and C-containing alleles, whereas circulating levels varied according to genotype in the same direction, but to a lesser extent. This may reflect physiological compensatory mechanisms that serve to attenuate (but not abolish) the effect of differences in gene *IGFBP-3* transcription on circulating IGFBP-3 levels.

Both height and BMI influenced the relationship between the −202 *IGFBP3* genotype and the circulating IGFBP-3 level. The polymorphism was less related to levels in shorter or leaner individuals than in taller and larger subjects. These findings may relate to the fact that taller and larger individuals tend to have higher levels of GH and insulin, respectively, both of which have been reported to up-regulate *IGFBP3* expression (18), assuming that the genotype at the −202 locus influences promoter responsivity to these regulators. Our observations suggest that it may be worthwhile to extend these studies to children, in whom measurement of circulating IGFBP-3 has been used to evaluate GH deficiency (32, 33), as well as in models designed to predict the efficacy of GH therapy (34, 35). A significant problem in either clinical context has been the large interindividual variability (14) of this analyte, and we hypothesize that the statistical relationship between circulating IGFBP-3 and GH status may be improved by subgrouping children according to genotype.

Although the circulating IGF-I concentration has recently been reported to be positively related to the risk of developing certain common epithelial cancers, high circulating IGFBP-3 levels appear to attenuate risk and/or to be inversely related to risk (7–12). On the basis of experimental studies (36, 37), we had hypothesized that cell renewal kinetics are influenced by IGF bioactivity, which is related to *IGFBP3* expression and hence to the −202 *IGFBP3* polymorphism. The lack of a detectable association between genotype and colorectal cancer risk in our study population is not unexpected, given that the variation in circulating IGFBP-3 concentrations across quintiles of the controls in the study population is greater than the spread across genotypes (high-

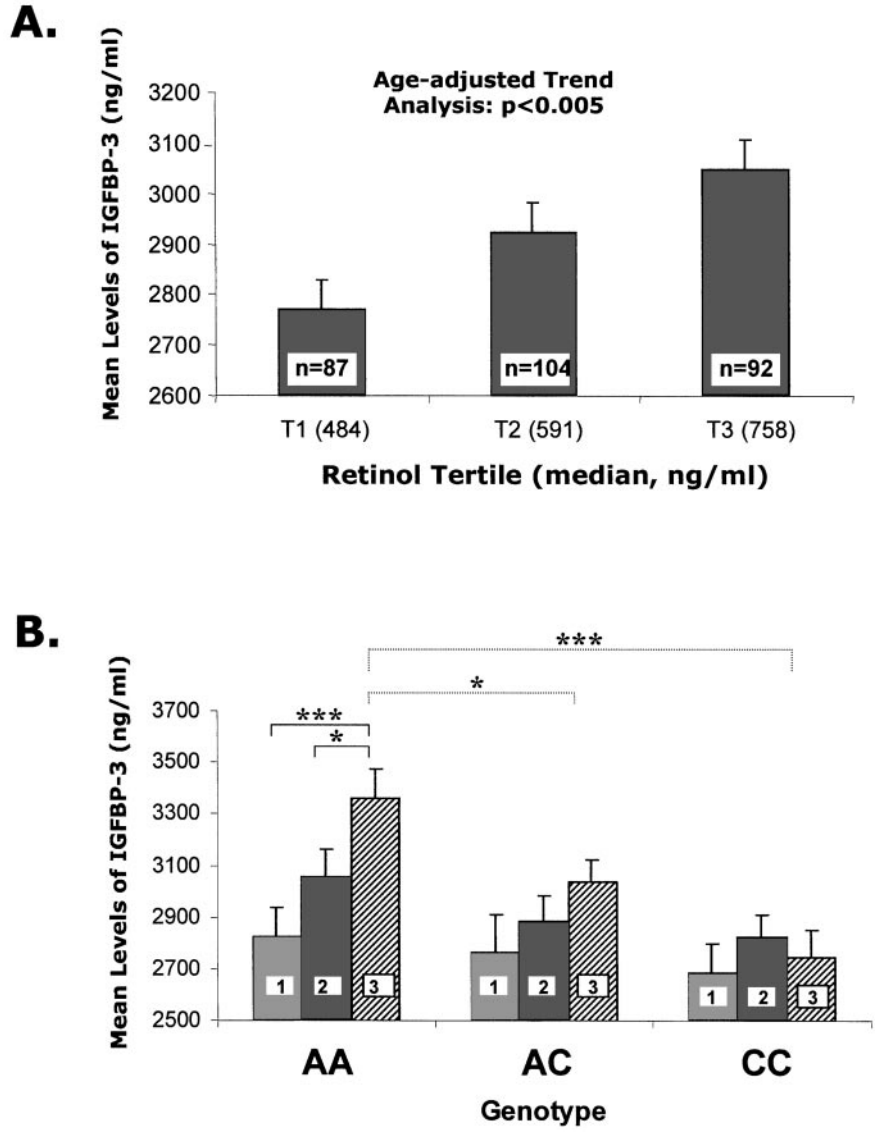

FIG. 4. Relationships among circulating IGFBP-3 levels, circulating serum retinol levels, and the −202 *IGFBP3* polymorphism. A, Mean IGFBP-3 levels according to tertile of retinol. The number of individuals (n) analyzed is indicated for each bar. B, Mean IGFBP-3 levels stratified by both genotype at nucleotide −202 and circulating retinol tertile. The small numbered boxes refer to retinol tertiles: 1, lower tertile (median, 484 ng/mL); 2, middle tertile (median, 591 ng/mL); and 3, upper tertile (median, 758 ng/mL). The *asterisks over the stippled lines* indicate the significant *P* values for differences in IGFBP-3 levels according to genotype for individuals within the upper retinol tertile (***, $P < 0.001$; *, $P < 0.05$). The *asterisks over the solid lines* indicate the significant *P* values for differences in IGFBP-3 levels between retinol tertiles for individuals with the AA genotype (***, $P < 0.001$; *, $P < 0.05$). All data are shown as the mean ± SE.

TABLE 2. Mean circulating IGFBP-3 concentrations according to BMI and IGFBP-3 genotype

BMI (kg/m^2)	Genotype (n)	Mean IGFBP-3 ± SE (ng/mL)	*P* value[a]
<23	AA (19)	3123 ± 128	
	AC (54)	2961 ± 76	NS
	CC (34)	2744 ± 96	0.02
23–24.9	AA (38)	3244 ± 91	
	AC (70)	3019 ± 67	0.05
	CC (39)	2894 ± 89	0.006
25–26.9	AA (32)	3038 ± 99	
	AC (59)	3032 ± 73	NS
	CC (42)	2792 ± 86	0.06
≥27	AA (20)	3524 ± 125	
	AC (44)	3009 ± 84	0.0007
	CC (27)	2683 ± 107	0.0001

[a] *P* values for age-adjusted IGFBP-3 levels within four BMI categories, AA *vs.* AC or CC.

est quintile mean IGFBP-3 = 3944 ng/mL; lowest quintile mean IGFBP-3 = 2161 ng/mL; mean IGFBP-3 for controls with AA genotype = 3274 ng/mL; mean IGFBP-3 for controls with CC genotype = 2753 ng/mL). We previously presented evidence that the RR for colorectal cancer of subjects in the highest quintile of circulating IGFBP-3 is 0.47 of that in subjects in the lowest (9). As we now calculate that 7.7% of the interindividual variability in circulating IGFBP-3 is attributable to the polymorphism, we would expect the RR related to the polymorphism to be small. Our sample size does not provide sufficient power to document small differences in relative risk or to detect a relationship between genotype and risk confined to subpopulations defined by either known modulators of IGFBP-3 levels or known colorectal cancer risk factors. The −202 *IGFBP3* locus, therefore, deserves investigation not as a rare mutation associated with a large increase in cancer risk, but, rather, as a site of common polymorphic variation that may subtly influence cancer risk. It is important to recognize that disease burden attributable to common polymorphisms that weakly predispose is not nec-

TABLE 3. Mean circulating IGFBP-3 concentrations according to height and IGFBP-3 genotype

Median height (cm)	Genotype (n)	Mean IGFBP-3 ± SE (ng/mL)	*P* value[a]
<173.0	AA (38)	3103 ± 91	
	AC (63)	3117 ± 70	NS
	CC (34)	2840 ± 96	0.046
173.0–176.9	AA (13)	2949 ± 155	
	AC (35)	2951 ± 95	NS
	CC (26)	2812 ± 110	NS
177.0–181.7	AA (30)	3377 ± 102	
	AC (61)	2969 ± 72	0.001
	CC (39)	2748 ± 89	0.0001
>181.8	AA (28)	3312 ± 106	
	AC (68)	2966 ± 68	0.006
	CC (43)	2769 ± 85	0.0001

[a] *P* values for age-adjusted IGFBP-3 levels within height categories, AA *vs.* AC or CC.

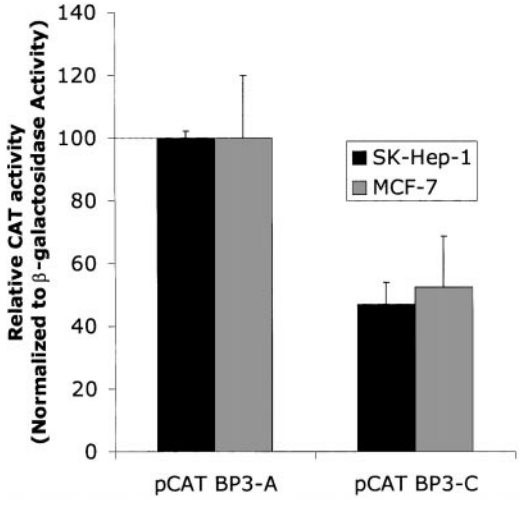

FIG. 5. Transient transfections of *IGFBP3* promoter fragment in a CAT reporter gene construct. Plasmids contained the *IGFBP3* minimal promoter and either the A (pCAT BP3-A) or C (pCAT BP3-C) nucleotide at position −202. Reporter gene activity was first normalized to β-galactosidase activity to control for transfection efficiency, and the activity of the pCAT BP3-C was expressed relative to that of pCAT BP3-A for each cell line. Transfections were performed in triplicate for each cell line, and data are shown as the mean ± SD.

essarily less than the burden attributable to rare mutations that strongly predispose.

It will be of interest to study racial variation in allele frequency at the −202 *IGFBP3* locus, as this may be related to recent observations that Afro-Americans, who have a higher risk and/or worse prognosis for both prostate cancer and breast cancer than Caucasians (38, 39), tend to have lower circulating IGFBP-3 levels (40, 41). We have determined that the −202 *IGFBP3* polymorphism is related to the circulating IGFBP-3 level in young women as well as men (41a).

The physiological basis for the positive relationship between circulating serum IGF-I level and cancer risk and the negative relationship between circulating IGFBP-3 level and cancer risk (7–12, 42) requires further investigation. These variables are positively correlated with each other, as IGFBP-3 is the major carrier protein for IGFs and thus is a major determinant of circulating IGF levels, but is oppositely related to risk. We speculate that circulating levels may not be direct determinants of risk, but, rather, may serve as proxies for tissue expression of these proteins, and that the balance between IGFs and IGFBPs in the microenvironment of normal and/or partially transformed epithelial cell populations may influence renewal kinetics and thus the risk of developing a clinically detectable cancer. It will be of interest to determine whether circulating IGFBP-3 levels and/or genotype at the −202 locus are related to tissue expression of IGFBP-3 and/or to rates of proliferation and apoptosis.

Candidate drugs for cancer prevention, such as antiestrogens, antiandrogens, and retinoids, appear to act in large part by reducing cellular proliferation in at-risk tissues. There is evidence that the antiproliferative action of these compounds is mediated at least in part through up-regulation of *IGFBP3* expression (20, 21, 23, 27). Indeed, a breast cancer prevention trial (43) has provided evidence that fenretinide (a synthetic derivative of retinoic acid) may reduce the risk of second breast cancers in premenopausal breast cancer survivors, in whom it increases plasma IGFBP-3 levels (27). The positive correlation we observed between circulating retinol levels and circulating IGFBP-3 levels is consistent with prior *in vitro* evidence that retinoids up-regulate *IGFBP3* expression. Furthermore, the fact that the IGFBP-3-retinol relation is influenced by the −202 *IGFBP3* polymorphism raises the possibility that the −202 *IGFBP3* locus influences responsiveness to growth inhibitors whose action involves up-regulation of *IGFBP3*. This justifies studies to evaluate the possibility that the −202 *IGFBP3* locus influences the efficacy of various agents proposed for cancer chemoprevention. There is a strong clinical need to define subpopulations for whom specific chemoprevention strategies are more or less likely to be effective, so as to optimize the value of such interventions from a risk/benefit point of view. *IGFBP3* provides an example of a gene whose polymorphic variation may be relevant to the pharmacogenomics of cancer prevention.

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