Longitudinal Study of Insulin-like Growth Factor, Insulin-like Growth Factor Binding Protein-3, and their Polymorphisms: Risk of Neoplastic Progression in Barrett's Esophagus

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Abstract

Background: **Insulin-like growth factor-I (IGF-I) is a potent mitogen. IGF-I and its main binding protein, IGF binding protein-3 (IGFBP-3), and their polymorphisms have been investigated in relation to risk of many cancers, but not esophageal adenocarcinoma.**
Materials and Methods: **We used data and specimens from a longitudinal study of persons with Barrett's esophagus (n = 344; median, 5.4 years follow up) to determine whether baseline serum concentrations of IGF-I and IGFBP-3 and associated polymorphisms were related to the risk of developing esophageal adenocarcinoma or flow cytometric abnormalities.**
Results: **Overall, circulating concentrations of IGF-I and IGBP-3 were not associated with risk of esophageal adenocarcinoma or flow cytometric abnormalities, with the exception of an approximate tripling of risk of aneuploidy among participants with higher IGFBP-3 levels [above median; adjusted hazard ratio (HR) comparing subjects with levels lower than median versus higher of equal to median, 2.7; 95% confidence interval (95% CI), 1.2-6.0; P = 0.01]. Genotypic analyses revealed that persons with the *IGF-I* [cytosine-adenine (CA)]$_{19}$ or the *IGFBP-3* A-202C C allele were associated with lower circulating concentrations of IGF-I (P_{trend} = 0.01) and IGFBP-3 (P_{trend} = 0.002), respectively. Persons with two copies of the *IGF-I receptors* 2-bp deletion allele had a nonsignificant 2-fold increased risk of tetraploidy (HR, 2.3; 95% CI, 0.9-5.9; P_{trend} = 0.11). After adjustment for IGFBP-3 levels, participants carrying two *IGFBP-3* C alleles had a significantly higher risk of developing aneuploidy (HR, 3.8; 95% CI, 1.0-14.0; P_{trend} = 0.04) than carriers of A alleles; whereas no associations were observed between the outcomes studied and the IGF-I receptors AGG trinucleotide repeat polymorphism at position 97.**
Conclusion: **Our findings, although based on a relatively small number of outcomes and subject to several limitations, indicate a potential role of the complex IGF system in neoplastic progression among persons with Barrett's esophagus.** (Cancer Epidemiol Biomarkers Prev 2007;16(11):2387–95)

Introduction

A rapid increase in the incidence of esophageal adenocarcinoma has occurred in Europe, Australia, Canada, and United States over the last three decades (1). White males have the highest incidence rate, followed by White females and Black males (2). Although the pathogenesis underlying causes are not well-understood, obesity is thought to play an important role (3).

Most esophageal adenocarcinoma arise in Barrett's esophagus, a metaplastic condition that develops in ~10% of persons who have chronic gastresophageal reflux disease. This disease affects ~40% of American adults (4). Each year, ~0.5% to 1% of persons with Barrett's esophagus progress to esophageal adenocarcinoma, a rate that is 30 to 40 times higher than that in the general population (5-10). Recent research has indicated that abnormalities, such as aneuploidy and tetraploidy, identify a particular high-risk subset of Barrett's esophagus patients (11-13). Given the large number of persons affected by Barrett's esophagus, which is estimated to be more than 1 million in the United States (14, 15), further research into methods to identify high-risk persons is warranted, so that effective primary and secondary prevention measures can be undertaken.

Insulin-like growth factors (IGF), such as IGF-I, are mitogens that play pivotal roles in the regulation of cell proliferation, differentiation, and apoptosis (16). Several epidemiologic studies have examined serum concentrations of IGF in relation to cancer risk (16, 17). For instance, in a recent metaanalysis, associations between IGF-I and prostate, colorectal, and premenopausal breast cancers were detected (18). The major binding protein of IGF-I,

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IGFBP-3, may also have a role in cancer progression (18, 19). It seems that IGFBP-3 has two potentially opposing roles in its influence on tumor behavior. It might have antiproliferative and proapoptotic properties by sequestering IGFs away from the IGF-I receptor (IGF-IR; ref. 20). Alternatively, IGFBP-3 interactions with cell or matrix components may concentrate IGF-I near their receptor, enhancing IGF-I activity (20). In some cellular environments, it might be antiapoptotic and act in an IGF-I–independent manner (21). Epidemiologic studies of the association between serum IGFBP-3 concentrations and cancer risk have also produced inconclusive results. It is hypothesized that some of these inconsistencies may in part be related to differences in the specificity of IGFBP-3 assays and measurement of functional versus total IGFBP-3 concentrations (22, 23). Measurements of total IGFBP-3 can include the detection of proteolytic IGFBP-3 fragments with lost affinity for IGF-I (23).

A highly polymorphic CA repeat polymorphism is located ~1 kb 5′ from the transcription start site of *IGF-I* on chromosome 12q12 (24). Studies relating *IGF-I* polymorphisms and circulating IGF-I blood concentrations have not been conclusive (25-28) nor were studies of polymorphisms and cancer risk (26, 29). *IGFBP-3* is located on chromosome 7p14-12. Polymorphism A-202C (rs2854744) has been associated with variation in serum IGFBP-3 concentrations (27, 30). This polymorphism has been shown to account for 6% to 9% of the variation in the circulating IGFBP-3 concentrations (31, 32). This *IGFBP-3* polymorphism has also been studied in relation to a number of cancers (33-36). IGF-IRs are located on the cell membrane. The most commonly reported polymorphisms in human *IGF-IR* include AGG trinucleotide repeat at position 97 (37) and 2-bp deletion in the 3′ untranslated region (38). To our knowledge, the aforementioned *IGF-IR* polymorphisms and their functional significance have not been studied in relation to esophageal adenocarcinoma.

We hypothesized that increased serum concentrations of IGF-I and decreased serum concentrations of IGFBP-3 and polymorphisms in *IGF-I, IGFBP-3*, and *IGF-IR* are associated with progression to aneuploidy, tetraploidy, or esophageal adenocarcinoma among patients with Barrett's esophagus. To investigate this hypothesis, we conducted a longitudinal study and determined baseline concentrations of serum IGF-I and IGFBP-3 and genotypes for polymorphisms in *IGF-I, IGFBP-3*, and *IGF-IR*.

Materials and Methods

Study Population, Biological Samples, and Disease End-Point Assessment. This was an ancillary study to the Seattle Barrett's Esophagus Predictors Research Cohort, which began in 1983. The participants in this cancer surveillance program had periodic endoscopies and multiple biopsies. Frequency of follow-ups ranged from every 6 months (for patients with high-grade dysplasia) to every 2 to 3 years (for patients without high-grade dysplasia). In addition, subjects with initial diagnosis of high-grade dysplasia undertook endoscopy at baseline, 1 month, and 3 months to rule out possible coexisting esophageal adenocarcinoma. Since February 1, 1995, new and ongoing participants donated blood samples; underwent an extensive personal interview about medical history, diet, and medication history; and provided anthropometric measurements (i.e., weight, height, and circumference of waist, abdomen, hips, and thigh; refs. 39, 40). The study was approved by the institutional review boards of University of Washington and Fred Hutchinson Cancer Research Center. Participants provided written informed consent.

Participants included in this analysis had baseline and follow-up assessments between February 1, 1995, and November 1, 2003. Eligible participants (n = 344) were those who had at least one follow-up at least 3 months after the baseline evaluation. Participants fasted overnight before donating blood samples. Serum, plasma, and WBC were obtained and divided into multiple aliquots and stored in cryovials at −70°C.

All participants underwent structured interviews at baseline given in person by trained staff. Interviews usually occurred in the clinic before endoscopy or occasionally in the participant's home. The baseline interview took ~45 min to complete and included medical history, family history of cancer and gastrointestinal disorders, past and current use of drugs, past and current tobacco and alcohol use, current occupation, and demographic characteristics. Height, weight, and anthropometric measurements were taken by the interviewers at baseline and at follow-up by use of a standard protocol (39, 40).

Methods of endoscopy, biopsy, and flow cytometry have been described elsewhere (11, 13). For most participants, four-quadrant biopsies were obtained from every alternate centimeter of Barrett's esophagus. For participants with a history of high-grade dysplasia, biopsies were taken every centimeter. Biopsy sampling was done at every endoscopy. One half of an endoscopic biopsy tissue sample from every 2 cm of the Barrett's segment was frozen, stored at −80°C in tissue culture medium with 5% FCS, 5 mmol/L HEPES buffer (pH 7.4), and 10% DMSO, and used for flow cytometry. The corresponding half was fixed for histologic interpretation. The remaining endoscopic biopsy tissue samples were placed in either Hollande's solution (until June 2001) or formalin (after July 2001) for subsequent histologic examination. Biopsies were interpreted by two university-based pathologists experienced in Barrett's esophagus field, both of whom were blinded to the flow cytometric and genetic findings. Participants were classified histologically according to the most advanced histology present in any biopsy sample. Flow cytometry histograms were interpreted by a single investigator, who was masked to the histologic or genetic results. Tetraploidy (abnormal 4N fraction) was defined by the presence of more than 6% of cells with a DNA content between 3.85 and 4.10N (11). A diagnosis of aneuploidy was made if the aneuploidy peak included at least 2.5% of cells in the biopsy sample and if discrete peaks were recorded on the histogram, showing aneuploid and diploid cell populations (11, 13, 41). A patient was classified as having aneuploidy or tetraploidy if the particular abnormality was noted for one or more biopsies from a given endoscopy.

Determination of Serum IGF-I and IGFBP-3 Concentrations. Concentrations of serum IGF-I and IGFBP-3 were measured using two-site immunoradiometric assay

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kits from Diagnostic Systems Laboratories, Inc. Before the determination of IGF-I concentration by immunoradiometric assay, the serum sample was treated with acid-ethanol to release IGF-I from its binding proteins. In addition to kit controls, aliquots of in-house serum quality control pools were included in each run to monitor assay performance. The assay for IGF-I had an intraassay CV of 3% to 4% and an interassay CV of 3% to 7%. For IGFBP-3, the intra- and interassay CV ranged from 2% to 6%.

Polymorphism Analyses. DNA was purified from peripheral blood WBC using standard methods (42). For the study of *IGF-I* CA repeat, *IGF-IR* AGG repeat, and IGF-IR 2-bp deletion, we generated PCR fragments containing these polymorphic loci and analyzed pooled PCR products on an ABI 310 genetic analyzer (Applied Biosystems, Inc.) to determine genotypes. For the *IGF-I* CA repeats, we used primers described by Rosen et al. (25). A 20-μL PCR reaction contained 25 ng genomic DNA, 0.1 μmol/L of each primer (forward primer was labeled with fluorescent label TET), 150 μmol/L of each deoxynucleotide triphosphate, and 0.8 units Taq polymerase in 1× PCR buffer. The thermocycling conditions included initial denaturation at 95°C for 2 min; 35 cycles of 94°C for 45 s, 60°C for 30 s, and 72°C for 1 min; and a final extension at 72°C for 5 min. To analyze the *IGF-IR* AGG repeats, we used primers described by Meloni et al. (37). The PCR reaction mix was identical to the IGF-I CA repeat protocol except that we used 200 μmol/L of each deoxynucleotide triphosphate. The thermocycles included 94°C for 3 min; 35 cycles of 94°C for 30 s, 67°C for 30 s, and 72°C for 30 s; and one cycle at 72°C for 5 min. Positive controls for both assays are purified and sequenced plasmid clones containing 18, 20, and 21 CA repeats or six and seven AGG repeats. Negative controls were prepared identically, but without DNA template. We amplified the 2-bp deletion of the *IGF-IR* gene according to Poduslo et al. (38). The forward and reversed primers (IGF-IRF1 5′GCT GAG GGA GGA GGC GGC3′-TET, IGF-IRR1 5′GGC GAG GGG CAG AAA CGC3′) amplify a 309-bp or 311-bp product. The PCR mix was identical to that for the IGF-I CA repeat protocol except the use of 100 μmol/L of each deoxynucleotide triphosphate. The thermocycles included initial denaturation at 94°C for 2 min; 8 cycles of 94°C for 45 s, 64°C for 30 s, and 72°C for 45 s; 27 cycles of 94°C for 45 s, 60°C for 30 s, and 72°C for 45 s; and a final extension at 72°C for 5 min. Positive controls were sequenced in-house genomic DNA of known genotypes. Negative controls contained no DNA template. Pooled PCR products were mixed with TAMRA size standard and denatured with formamide at 95°C for 2 min and analyzed on the ABI 310. Sample peaks were compared with known peaks from the GeneScan-500 size standard and were assigned relative mobility units via the ABI GeneScan software. The ABI Genotyper software assigned a repeat length to each peak based on relative mobility unit bin sizes determined with cloned and sequenced plasmid standards with known number of repeats or with sequenced in-house DNA with or without the 2-bp deletion.

We determined the *IGFBP-3* A-202C polymorphism by PCR/RFLP using primers described by Deal et al. (43). A 20-μL PCR contained 25 ng genomic DNA, 0.2 μmol/L of each primer, 100 μmol/L of each deoxynucleotide triphosphate, 2.5 mmol/L Mg^{2+}, 1× Q-Solution, 0.84 units Taq polymerase in 1× PCR buffer. The thermocycling variables included initial denaturation at 95°C for 2 min; 5 cycles of 95°C for 45 s, 66°C for 30 s, and 72°C for 1 min; 35 cycles of 94°C for 45 s, 60°C for 30 s, 72°C for 1 min; and a final extension at 72°C for 5 min. Positive controls were in-house DNA samples of known genotypes; template-free reactions were used for negative controls. The PCR products were digested with *Bsi*HKA I and resolved on a 2.5% agarose gel. *Bsi*HKA I restriction generated 162 and 242 bp bands for A/A individuals, and 162 and 289 bp bands for C/C homozygotes. Heterozygotes showed all bands.

Statistical Analyses. On the basis of baseline serum concentrations of IGF-I and IGFBP-3 and their median splits, participants (n = 344) were classified and studied for the development of any of the outcomes. Cox proportional hazards models were used to calculate hazard ratios (HR) and 95% confidence interval (95% CI), adjusting as necessary for gender, smoking status, age (<45.0, 45.0-54.9, 55.0-64.9, 65.0-74.9, and >75.0 years), and waist-to-hip ratio (quartile ranges: <0.912, 0.912-.0.957, 0.958- 0.995, and >0.995). Participants were initially classified as ever smokers (≥1 cigarette a day for ≥6 months) or nonsmokers. In the analysis, we used three categories of smoking status: current (at baseline), former smoker, and nonsmoker. Other variables examined for possible confounding included education (high-school graduate or less, attended college without graduating, and college graduate), NSAID use, and selenium concentration (quartile ranges: <1.54, 1.54-1.70, 1.71- 1.84, and >1.84 μmol/L), and body mass index (categories: <25.0, 25.0-27.4, 27.5-29.9, and >29.9 kg/m^2). NSAID categories included current users (defined as those who had used NSAID at least weekly for ≥6 months at the time of, or within a year of, baseline interview), former users (defined as those who had used NSAID at least weekly for ≥6 months, but not within a year of baseline interview), or never users (defined as those who had never used NSAID at least weekly for ≥6 months). Possible interactions between IGFs and NSAID and waist-to-hip ratio were tested using likelihood ratio tests. Subgroup analyses of ethnic origin were not possible because most participants [327 of 340 (96%) with known ethnic origin] identified themselves as White. We also calculated partial Pearson correlation coefficient to test for correlation between serum concentrations of IGF-I and IGFBP-3.

The three end points were considered to develop independently from each other and were analyzed separately. All persons in the cohort were cancer-free at baseline. Risk of aneuploidy was assessed among persons who were aneuploidy-free at baseline, regardless of their tetraploidy status, and vice versa. Development of esophageal adenocarcinoma indicated the end of follow-up for this report; persons developing esophageal adenocarcinoma were considered to also have developed aneuploidy and tetraploidy as well. IGF-I and IGFBP-3 concentrations were tested in median splits, as well as tertiles, quartiles, and continuous variables with little difference from median split categories. Due to sample

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Table 1. Observation time and the development of esophageal adenocarcinoma or its precursor lesions

	*n**	Person-years	Median follow-up, y (range)	No. events
Cancer	344	1,637	5.4 (0.2-8.9)	38
Aneuploidy	309	1,467	5.5 (0.4-8.9)	35
Tetraploidy	309	1,431	5.3 (0.2-8.9)	42

*Number of subjects without the outcome at baseline.

size limitation, the data are presented in median splits. We also explored a continuous (grouped linear) model for genotypes as well as categorical, but did not observe importantly different patterns of results.

Genotype-Phenotype Association. ANOVA (*F* statistics) was used to compare the serum concentrations of IGF-I and IGFBP-3 within genotype categories. Multiple regression models were also fitted with gender, smoking status, quartiles of body mass index and waist-to-hip ratio, and age categories. Adjusted and unadjusted mean serum concentrations of IGF-I by genotype categories (as listed in Table 5) were determined using the Stata adjust command.

Genotype-Disease Risk Association. Three hundred forty-three participants were classified and studied as above with regard to *IGFBP-3*, *IGF-I*, and *IGF-IR* genotypes and disease outcomes. All statistical tests were two-sided. We assessed departures from Hardy-Weinberg disequilibrium by testing the difference between the observed (sampled) and expected genotype frequencies under Hardy-Weinberg equilibrium and did asymptotic Hardy-Weinberg equilibrium tests (44) using Stata genhw and genhwi commands.

We used several methods to test for the appropriateness of the proportional hazards assumption and other potential misspecifications of our model. Such methods included partial likelihood ratio tests for the significance of interaction terms between IGFs and other risk factors and a test for a relation between scaled Schoenfeld residuals and time. None of these methods showed any significant misspecifications or violations of the assumptions in the model. Statistical analyses were done using Stata software version 9.1.

Results

The 344 participants were followed for a median of 5.4 years (Table 1). The mean number of follow-ups for cancer, aneuploidy, and tetraploidy analyses were 3.7, 3.2, and 3.2, respectively (number of follow-ups for cancer analysis ranged from 1 to 20). The mean age for participants whose serum IGF-I concentrations were determined was 61.6 years (SD, 11.7 years). The number of events and follow-up times are presented in Table 1. Characteristics of the participants and the mean serum concentrations of IGF-I and IGFBP-3

Table 2. Selected characteristics of participants and serum IGF-I and IGFBP-3 concentrations (at baseline)

	*n**	IGF-I (ng/mL), mean (SD)	IGFBP-3 (ng/mL), mean (SD)
Age (y)			
<55	108 (31%)	231 (90)	3,991 (811)
>55	236 (69%)	175 (77)	3,458 (854)
Gender			
Male	278 (81%)	202 (82)	3,586 (854)
Female	66 (19%)	153 (89)	3,789 (946)
Race			
White	327 (96%)	194 (85)	3,637 (869)
Non-White	13 (4%)	169 (97)	3,250 (1,009)
Education			
<College	94 (27%)	187 (87)	3,581 (937)
≥College	250 (73%)	195 (85)	3,642 (852)
Cigarette smoking			
Never	117 (34%)	205 (91)	3,716 (950)
Former	191 (56%)	191 (82)	3,570 (813)
Current	36 (10%)	163 (82)	3,622 (938)
Body mass index (kg/m^2)			
<28.7	171 (50%)	193 (88)	3,561 (876)
>28.7	171 (50%)	193 (83)	3,686 (876)
Waist/hip			
<0.96	170 (50%)	194 (26)	3,656 (881)
>0.96	173 (50%)	192 (83)	3,595 (873)
Selenium levels (μmol/L)			
<1.71	177 (51%)	186 (84)	3,529 (851)
>1.71	167 (49%)	201 (86)	3,727 (890)
NSAID use			
Never	143 (42%)	201 (86)	3,687 (843)
Former	73 (21%)	190 (89)	3,724 (908)
Current	125 (37%)	186 (83)	3,495 (891)
Total subjects	344	193 (86)	3,625 (875)

*Number of subjects may not add up to 344 due to missing values for some variables.

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within each subgroup are shown in Table 2. Serum concentrations of IGF-I were found to be correlated strongly with those of IGFBP-3 (partial Pearson correlation coefficient, 0.59; $P < 0.001$). Both concentrations showed a decrease with increasing age, but little variation by other risk factors. IGF-I serum levels were higher in men than in women (partial Pearson correlation coefficient, 0.23; $P < 0.0001$), whereas serum levels of IGFBP-3 were lower in men (partial Pearson correlation coefficient, −0.09; $P = 0.09$). The association between serum IGF-I and IGFBP-3 concentrations, categorized into quartiles, and disease outcomes are presented in Table 3. There was a suggestion of increased risk of esophageal adenocarcinoma with higher concentrations of IGF-I and IGF-I/IGFBP-3 molar ratio. Higher concentrations of IGFBP-3 were associated with a significantly increased risk of aneuploidy ($P_{trend} = 0.02$), but not esophageal adenocarcinoma or tetraploidy. No significant interactions were noted between IGF-I or IGFBP-3 concentrations and participant age, gender, NSAID use, waist-to-hip ratio, or serum selenium concentrations in association with the outcomes. Continuous models for IGF-I and IGFBP-3 were not associated with the outcomes. We did not find any evidence of departure from Hardy-Weinberg equilibrium in the genotype frequencies among our participants (*IGFBP-3* −202 A/C, $P = 0.82$; *IGF-I* CA repeats, $P = 0.37$; *IGF-IR* AGG repeats, $P = 0.77$; and *IGF-IR* 2-bp deletion, $P = 0.77$).

The number of CA repeats in *IGF-I* ranged from 11 to 24 (data not shown) with the $(CA)_{19}$ allele being the most common (64%), followed by $(CA)_{20}$ at 19%. For *IGFBP-3* A-202C polymorphism, the C allele (56%) had a higher frequency than the A allele. For *IGF-IR*, the $(AGG)_7$ (58%) and $(AGG)_6$ (41%) alleles were the most common (range of repeats, 5-8), and ∼30% of the study participants carried a 2-bp deletion in *IGF-IR*.

The *IGF-I* $(CA)_{19}/(CA)_{19}$ genotype was associated with lower IGF-I serum concentrations than *IGF-I* genotypes with non-19 repeats ($P_{trend} = 0.04$, after adjusting for age and gender $P_{trend} = 0.01$; Table 4). Similarly, adjusted mean serum concentrations of IGFBP-3 were significantly lower ($P_{trend} = 0.002$) among carriers of *IGFBP-3* AC and CC genotypes.

There was a trend toward an association between presence of the *IGF-I* $(CA)_{19}$ allele (negatively) or the *IGF-IR* 2-bp deletion allele (positively) and the risk of esophageal adenocarcinoma and tetraploidy, respectively (Table 5). The aforementioned associations did not significantly change after adjusting for serum IGF-I concentrations. No association was observed between the studied polymorphisms in *IGFBP-3, IGF-IR*, and the risk of esophageal adenocarcinoma. The *IGFBP-3* C/C genotype showed a modest (but not significant) association with aneuploidy and tetrapoloidy. The former association became stronger and significant [the HRs comparing AC and CC with AA genotypes were 2.8 (95% CI, 0.8-9.4) and 3.8 (95% CI, 1.0-14.0), respectively; $P_{trend} = 0.04$] after adjusting for quartiles of serum concentration of IGF-I and IGFBP-3, waist-to-hip ratio, smoking status, gender, and age categories. We also observed a stronger positive association between serum concentrations of IGFBP-3 and risk of aneuploidy after adjustment for *IGFBP-3* genotypes (Table 6).

Discussion

We did not detect a significant association between circulating IGF-I concentrations or IGF-I/IGFBP-3 molar ratio and the development of esophageal adenocarcinoma or its precursors. However, after adjusting for major risk factors, we found that higher concentrations of IGFBP-3 were associated with an increased risk of aneuploidy, possibly suggesting a role for IGFBP-3 in early stages of cancer development. In analyses adjusting for IGFBP-3 along with major risk factors, we also found suggestive evidence that persons with the *IGFBP-3* C/C genotype may be at moderately elevated risk of developing flow cytometric abnormalities.

There are no other studies, to our knowledge, that report an association between IGFBP-3 or related

Table 3. HRs of esophageal adenocarcinoma and the precursor lesions by serum IGF-I, IGFBP-3, and their ratios and frequency distribution (%) among persons with Barrett's esophagus

	≤Median	>Median	P_{trend}*
IGF-I †			
Tetraploidy			
Yes (*n* = 42)	54.8	45.2	
No (*n* = 267)	49.8	50.2	
HR ‡	1.0	1.1 (0.6-2.3)	0.62
Aneuploidy			
Yes (*n* = 35)	45.7	54.3	
No (*n* = 274)	51.1	48.9	
HR ‡	1.0	0.9 (0.4-1.9)	0.74
Cancer			
Yes (*n* = 38)	42.1	57.9	
No (*n* = 306)	51.0	49.0	
HR ‡	1.0	1.3 (0.6-2.8)	0.32
IGFBP-3§			
Tetraploidy			
Yes (*n* = 42)	54.8	45.2	
No (*n* = 267)	50.6	49.4	
HR ‡	1.0	0.91 (0.5-1.9)	0.93
Aneuploidy			
Yes (*n* = 35)	31.4	68.6	
No (*n* = 274)	51.5	48.5	
HR ‡	1.0	2.7 (1.2-6.0)	0.02
Cancer			
Yes (*n* = 38)	42.1	57.9	
No (*n* = 306)	51.0	49.0	
HR ‡	1.0	1.3 (0.6-2.8)	0.61
IGF-I/IGFBP-3∥			
Tetraploidy			
Yes (*n* = 42)	59.5	40.5	
No (*n* = 267)	52.4	47.6	
HR ‡	1.0	1.0 (0.5-2.9)	0.60
Aneuploidy			
Yes (*n* = 35)	54.3	45.7	
No (*n* = 274)	52.2	47.8	
HR ‡	1.0	1.0 (0.7-1.4)	0.10
Cancer			
Yes (*n* = 38)	39.5	60.5	
No (*n* = 306)	53.3	46.7	
HR ‡	1.0	1.8 (0.9-359)	0.10

*P_{trend} values are based on quartile variables.
†IGF-I median (ng/mL), 186.159, additional adjustment for IGFBP-3 levels.
‡Adjusted for gender, smoking status (current, former, or nonsmoker), quartiles of age, and waist/hip ratios.
§IGFBP-3 median (ng/mL), 3,634.9, additional adjustment for IGF-I levels.
∥IGF-I/IGFBP-3 (molar ratio) median, 0.1909.

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Table 4. Polymorphisms in IGF-I and IGFBP-3 in relation to serum concentrations of their protein products at baseline

	n*	Arithmetic mean (SD)	Arithmetic mean (SD)
IGF-I (CA)$_{19}$ genotypes		IGF-I (ng/mL)	IGF-I (ng/mL), adjusted †
$(CA)_{19}/(CA)_{19}$	136 (40%)	180 (78)	178 (79)
$(CA)_{19}/(CA)_{non\text{-}19}$	166 (48%)	205 (87)	193 (79)
$(CA)_{non\text{-}19}/(CA)_{non\text{-}19}$	41 (12%)	190 (99)	209 (80)
P ‡		0.04	0.01
Overall mean		193 (86)	
IGFBP-3 −202 A/C genotypes		IGFBP-3 (ng/mL)	IGFBP-3 (ng/mL), adjusted †
AA	65 (19%)	3,897 (806)	3,819 (822)
AC	171 (50%)	3,611 (907)	3,602 (819)
CC	107 (31%)	3,478 (834)	3,384 (821)
P ‡		0.009	0.002
Overall mean		3624 (876)	

*Total n = 343.
†Adjustment for age and gender.
‡ANOVA (F statistics) for difference in means.

genotypes and esophageal adenocarcinoma. Other evidence supporting the role of IGFs in esophageal cancer development stem from studies of biological function and expression of the IGF system factors in esophageal cancer; IGF-I acts as a mitogen or growth stimulant in human esophageal cancer cell lines (45, 46) and IGFBP-3 mRNA is overexpressed in 80% of primary esophageal squamous cell carcinomas and 60% of primary esophageal adenocarcinoma (47).

We observed very little increase in the risk of esophageal adenocarcinoma among the subjects with higher than median serum concentrations of IGF-I [HR = 1.3 (95% CI, 0.6-2.8), comparing higher than median with the lower or equal; Table 3]. Given the lower serum concentrations of IGF-I among carriers of the $(CA)_{19}/(CA)_{19}$ genotype, we had expected to find a lower cancer risk in the $(CA)_{19}$ allele carriers. Interestingly, we found the opposite. This is similar to our finding showing that the *IGFBP-3* C/C genotype was associated with lower IGFBP-3 concentrations and a higher risk of aneuploidy. Our data support the hypothesis that the *IGF-I* $(CA)_{19}$ allele and the *IGFBP-3* C allele have effects independent of the circulating IGF-I and IGFBP-3 concentrations, respectively. However, the $(CA)_{19}$ allele effect is not as strong as the C allele in relation to the outcomes, possibly explaining why we did not observe a significant change in the $(CA)_{19}$ allele association with esophageal adenocarcinoma after adjustment for serum concentrations of IGF-I.

The frequencies of the $(CA)_{19}$ and the $(CA)_{20}$ alleles in our study (64% and 19%, respectively) are similar to previous reports (59-70% and 17%, respectively) among

Table 5. HRs of developing esophageal adenocarcinoma or its precursor lesions by polymorphisms of the IGF-I, IGBP-3, and IGF-IR genes among participants with Barrett's esophagus

	n*	Aneuploidy		Tetraploidy		Esophageal adenocarcinoma	
		No. cases/ no. controls	HR (95% CI)	No. cases/ no. control	HR (95% CI)	No. cases/ no. controls	HR (95% CI)
IGF-I (CA)$_{19}$ genotypes							
$(CA)_{19}/(CA)_{19}$	136 (40%)	13/111	1	17/111	1	16/120	1
$(CA)_{19}/(CA)_{non\text{-}19}$	166 (48%)	17/128	0.9 (0.5-2.3)	18/125	0.9 (0.8-1.8)	19/147	1.0 (0.5-1.9)
$(CA)_{non\text{-}19}/(CA)_{non\text{-}19}$	41 (12%)	5/34	1.1 (0.7-2.9)	7/30	1.1 (0.4-2.6)	3/38	0.5 (0.2-1.8)
P_{trend}			0.9		1.0		0.4
IGFBP-3 −202 A/C genotypes							
AA	65 (19%)	3/55	1	6/51	1	10/55	1
AC	171 (50%)	19/135	2.4 (0.7-8.2)	20/137	1.2 (0.5-3.0)	15/156	0.6 (0.3-1.3)
CC	107 (31%)	13/83	2.6 (0.7-9.1)	16/78	1.6 (0.6-4.1)	13/94	0.8 (0.4-1.9)
P_{trend}			0.2		0.3		0.8
IGF-IR genotypes							
AGG repeats							
7,7	111 (32%)	13/89	1	14/88	1	14/97	1
7,6	174 (51%)	15/140	0.7 (0.3-1.5)	19/137	0.9 (0.4-1.8)	20/154	0.9 (0.4-9.8)
6,6	54 (16%)	6/41	0.9 (0.3-2.3)	8/39	1.0 (0.4-2.5)	3/51	0.4 (0.1-1.4)
Others	4 (1%)	1/3	2.6 (0.3-19.7)	1/2	2.8 (0.4-21.7)	1/3	2.3 (0.3-17.5)
P_{trend}			0.9		0.8		0.3
2-bp deletion							
No deletion	167 (49%)	17/132	1	17/135	1	15/152	1
Heterozygote	143 (42%)	14/116	0.9 (0.5-1.9)	19/107	1.3 (0.7-2.5)	19/124	1.5 (0.8-3.0)
Homozygote	33 (9%)	4/25	1.4 (0.5-4.3)	6/24	2.3 (0.9-5.9)	4/29	1.5 (0.5-4.6)
P_{trend}			0.4		0.1		0.3

*Total number of cases and controls may not add up to 343 due to exclusion criteria in each end-point analysis.

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Table 6. Effect of adjusting for IGFBP-3 genotypes on HRs of developing aneuploidy for the quartiles of serum IGFBP-3 among persons with Barrett's esophagus

Biomarker	≤Median	>Median	P_{trend}*
IGFBP-3	1.0	2.7 (1.2-6.0)	0.02
IGFBP-3 (adjusted)†	1.0	3.3 (1.5-7.5)	<0.01

NOTE: HR, adjusted for age categories, gender, smoking status, and quartiles of waist/hip ratios and median splits of IGF-I.
*P_{trend} values are based on quartile variables.
†Additional adjustment for three categories of IGFBP-3 −202 A/C genotypes as listed in Table 5.

Caucasians (31, 48, 49). Allele frequencies for *IGFBP-3* A-202 C and *IGF-IR* 2-bp deletion polymorphisms were also similar to those reported for Caucasians (38, 50). Our finding that the $(CA)_{19}$ allele was related to a lower serum concentration of IGF-I is consistent with results from some (25, 26), but not all, studies (27, 51). Most previous studies adjusted for age or gender (31, 52). We found the *IGF-I* genotype-phenotype association had a clear trend after adjusting for age and gender (Table 4). Our results also support previous findings (27, 30) regarding the dose-dependent association between the presence of the *IGFBP-3* A-202C C allele and a lower circulating IGFBP-3 concentration.

There was no significant association observed between risk of esophageal adenocarcinoma, tetraploidy, or aneuploidy and the 2-bp deletion in *IGF-IR*, although there was suggestion of an increase in risk of tetraploidy and esophageal adenocarcinoma. Other studies have shown the presence of IGF-IR in the human esophageal epithelium (53) and suggested that IGF may influence the development of colorectal (54), renal (55), and prostate (56) cancers through an auto/paracrine loop. Our data do not provide strong support for a similar association in esophageal adenocarcinoma. The effect of the 2-bp deletion on the IGF-IR expression/function is not well studied. There is a report of an association between *IGF-IR* mutations and intrauterine and postnatal growth retardation (57).

An important strength of our study is its longitudinal design, in which concentrations of IGF-I and IGFBP-3 were determined using samples obtained before the development of flow cytometry abnormalities or cancer among patients with Barrett's esophagus. Another strength is our use of intermediate abnormalities (aneuploidy and tetraploidy) that have been validated (11-13) as strong predictors for risk of esophageal adenocarcinoma as outcomes. Finally, the availability of data for major known or suspected risk factors for esophageal adenocarcinoma enabled us to control for possible confounding factors.

Our study has a number of limitations. The free fraction of IGF-I, which is considered the biologically active fraction, was not directly measured in our study. Instead, we measured total serum IGF-I and estimated the free IGF-I concentrations by adjusting for IGFBP-3 serum concentrations or by using IGF-I/IGFBP-3 molar ratio as a surrogate. However, IGF-I/IGFBP-3 is shown not to be an accurate approximation for free fraction of IGF-I (58). Another important limitation is the relatively small number of events recorded thus far, although ours is one of the largest prospective studies of participants with Barrett's esophagus. This small number of events resulted in somewhat imprecise point estimates and limited our ability to identify variations in risk in different subgroups. The minimum detectable relative risk, with a power of 80%, in our study was 2.5 to 3.7 (varies with the type of outcome and predictor). In addition, the IGF system is complex and we were not able to measure all of the components in our study, such as IGF-IR expression (59) and insulin (a regulator of IGFBP synthesis; ref. 60). Thus, the unmeasured variables could confound the association between serum IGF-I or IGFBP-3 and disease progression. Our study was based on a single measurement of IGF-I and IGFBP-3 serum concentrations using samples that had been collected and stored at −70°C for up to 8 years before assay. However, it is unlikely that these characteristics have adversely influenced our results, since there is evidence for the lack of appreciable seasonal and diurnal variation of IGF (61) and the lack of significant changes in IGF and IGF-related proteins in frozen serum after 5 to 9 years of storage (62, 63).

We used time-to-detection in our analysis. In the low-risk group with the longest (every 24 months) follow-ups, outcomes may develop any time within the 2-year period. Therefore, the time-to-detection may have underestimated the incidence rate in this group. Also, some participants may have had the outcome at baseline, but we failed to detect them.

In summary, this longitudinal study is the first to provide evidence for an increased risk of neoplastic progression among persons with Barrett's who have higher concentrations of IGFBP-3 at baseline or are carriers of *IGFBP-3* C/C genotype. However, it does not support a relationship between IGF-I serum concentration and risk of esophageal adenocarcinoma. These findings, which should be considered preliminary in nature as they are based on small numbers and subject to several limitations, indicate a potential role of the complex IGF system in neoplastic progression among persons with Barrett's esophagus. However, a more comprehensive study with a larger number of events and the measurement of other key factors, such as IGF-IR, functional fraction of IGFBP-3, insulin, and diet, is needed to further delineate the possible role of the IGF system on esophageal adenocarcinoma progression.

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References

1. Bollschweiler E, Wolfgarten E, Gutschow C, Holscher AH. Demographic variations in the rising incidence of esophageal adenocarcinoma in White males. Cancer 2001;92:549–55.
2. Kubo A, Corley DA. Marked regional variation in adenocarcinomas of the esophagus and the gastric cardia in the United States. Cancer 2002;95:2096–102.
3. Chow WH, Blot WJ, Vaughan TL, et al. Body mass index and risk of

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adenocarcinomas of the esophagus and gastric cardia. J Natl Cancer Inst 1998;90:150–5.

4. Morales TG, Sampliner RE. Barrett's esophagus: update on screening, surveillance, and treatment. Arch Intern Med 1999;159:1411–6.
5. Drewitz DJ, Sampliner RE, Garewal HS. The incidence of adenocarcinoma in Barrett's esophagus: a prospective study of 170 patients followed 4.8 years. Am J Gastroenterol 1997;92:212–5.
6. Hameeteman W, Tytgat GN, Houthoff HJ, van den Tweel JG. Barrett's esophagus: development of dysplasia and adenocarcinoma. Gastroenterology 1989;96:1249–56.
7. Miros M, Kerlin P, Walker N. Only patients with dysplasia progress to adenocarcinoma in Barrett's oesophagus. Gut 1991;32:1441–6.
8. O'Connor JB, Falk GW, Richter JE. The incidence of adenocarcinoma and dysplasia in Barrett's esophagus: report on the Cleveland Clinic Barrett's Esophagus Registry. Am J Gastroenterol 1999; 94:2037–42.
9. Robertson CS, Mayberry JF, Nicholson DA, James PD, Atkinson M. Value of endoscopic surveillance in the detection of neoplastic change in Barrett's oesophagus. Br J Surg 1988;75:760–3.
10. van der Burgh A, Dees J, Hop WC, van Blankenstein M. Oesophageal cancer is an uncommon cause of death in patients with Barrett's oesophagus. Gut 1996;39:5–8.
11. Rabinovitch PS, Longton G, Blount PL, Levine DS, Reid BJ. Predictors of progression in Barrett's esophagus III: baseline flow cytometric variables. Am J Gastroenterol 2001;96:3071–83.
12. Reid BJ, Prevo LJ, Galipeau PC, et al. Predictors of progression in Barrett's esophagus II: baseline 17p (p53) loss of heterozygosity identifies a patient subset at increased risk for neoplastic progression. Am J Gastroenterol 2001;96:2839–48.
13. Reid BJ, Levine DS, Longton G, Blount PL, Rabinovitch PS. Predictors of progression to cancer in Barrett's esophagus: baseline histology and flow cytometry identify low- and high-risk patient subsets. Am J Gastroenterol 2000;95:1669–76.
14. Cameron AJ. Epidemiologic studies and the development of Barrett's esophagus. Endoscopy 1993;25:635–6.
15. Vaughan T. The esophagus. In: EL Franco, TW Rohan, editors. Cancer precursorss: epidemiology, detection and prevention. 2002. p. 96–116.
16. Khandwala HM, McCutcheon IE, Flyvbjerg A, Friend KE. The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev 2000;21:215–44.
17. Yu H, Jin F, Shu XO, et al. Insulin-like growth factors and breast cancer risk in Chinese women. Cancer Epidemiol Biomarkers Prev 2002;11:705–12.
18. Renehan AG, Zwahlen M, Minder C, et al. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 2004;363:1346–53.
19. Harman SM, Metter EJ, Blackman MR, et al. Serum levels of insulin-like growth factor I (IGF-I), IGF-II, IGF-binding protein-3, and prostate-specific antigen as predictors of clinical prostate cancer. J Clin Endocrinol Metab 2000;85:4258–65.
20. Firth SM, Baxter RC. Cellular actions of the insulin-like growth factor binding proteins. Endocr Rev 2002;23:824–54.
21. Oh Y, Muller HL, Ng L, Rosenfeld RG. Transforming growth factor-β-induced cell growth inhibition in human breast cancer cells is mediated through insulin-like growth factor-binding protein-3 action. J Biol Chem 1995;270:13589–92.
22. Kaaks R, Rinaldi S, Lukanova A, et al. Correspondence re: Giovannucci et al., A prospective study of plasma insulin-like growth factor-1 and binding protein-3 and risk of colorectal neoplasia in women. Cancer Epidemiol Biomarkers Prev 2000;9: 345–9. Cancer Epidemiol Biomarkers Prev 2001;10:1103–4.
23. Rinaldi S, Kaaks R, Zeleniuch-Jacquotte A, et al. Insulin-like growth factor-I, IGF binding protein-3, and breast cancer in young women: a comparison of risk estimates using different peptide assays. Cancer Epidemiol Biomarkers Prev 2005;14:48–52.
24. Moschos SJ, Mantzoros CS. The role of the IGF system in cancer: from basic to clinical studies and clinical applications. Oncology 2002;63: 317–32.
25. Rosen CJ, Kurland ES, Vereault D, et al. Association between serum insulin growth factor-I (IGF-I) and a simple sequence repeat in IGF-I gene: implications for genetic studies of bone mineral density. J Clin Endocrinol Metab 1998;83:2286–90.
26. Jernstrom H, Sandberg T, Bageman E, Borg A, Olsson H. Insulin-like growth factor-1 (IGF1) genotype predicts breast volume after pregnancy and hormonal contraception and is associated with circulating IGF-1 levels: implications for risk of early-onset breast cancer in young women from hereditary breast cancer families. Br J Cancer 2005;92:857–66.
27. Wong HL, Delellis K, Probst-Hensch N, et al. A new single nucleotide polymorphism in the insulin-like growth factor I regulatory region associates with colorectal cancer risk in Singapore Chinese. Cancer Epidemiol Biomarkers Prev 2005;14: 144–51.
28. Morimoto LM, Newcomb PA, White E, Bigler J, Potter JD. Variation in plasma insulin-like growth factor-1 and insulin-like growth factor binding protein-3: genetic factors. Cancer Epidemiol Biomarkers Prev 2005;14:1394–401.
29. Morimoto LM, Newcomb PA, White E, Bigler J, Potter JD. Insulin-like growth factor polymorphisms and colorectal cancer risk. Cancer Epidemiol Biomarkers Prev 2005;14:1204–11.
30. Canzian F, McKay JD, Cleveland RJ, et al. Polymorphisms of genes coding for insulin-like growth factor 1 and its major binding proteins, circulating levels of IGF-I and IGFBP-3 and breast cancer risk: results from the EPIC study. Br J Cancer 2006;94:299–307.
31. Jernstrom H, Deal C, Wilkin F, et al. Genetic and nongenetic factors associated with variation of plasma levels of insulin-like growth factor-I and insulin-like growth factor- binding protein-3 in healthy premenopausal women. Cancer Epidemiol Biomarkers Prev 2001;10: 377–84.
32. Schernhammer ES, Hankinson SE, Hunter DJ, Blouin MJ, Pollak MN. Polymorphic variation at the -202 locus in IGFBP-3: Influence on serum levels of insulin-like growth factors, interaction with plasma retinol and vitamin D and breast cancer risk. Int J Cancer 2003;107: 60–4.
33. Zhang ZW, Newcomb PV, Moorghen M, et al. Insulin-like growth factor binding protein-3: relationship to the development of gastric pre-malignancy and gastric adenocarcinoma (United Kingdom). Cancer Causes Control 2004;15:211–8.
34. Wang L, Habuchi T, Tsuchiya N, et al. Insulin-like growth factor-binding protein-3 gene -202 A/C polymorphism is correlated with advanced disease status in prostate cancer. Cancer Res 2003;63: 4407–11.
35. Li L, Cicek MS, Casey G, Witte JS. No association between genetic polymorphisms in IGF-I and IGFBP-3 and prostate cancer. Cancer Epidemiol Biomarkers Prev 2004;13:497–8.
36. Slattery ML, Samowitz W, Curtin K, et al. Associations among IRS1, IRS2, IGF1, and IGFBP-3 genetic polymorphisms and colorectal cancer. Cancer Epidemiol Biomarkers Prev 2004;13:1206–14.
37. Meloni R, Fougerousse F, Roudaut C, Beckmann JS. Trinucleotide repeat polymorphism at the human insulin-like growth factor I receptor gene (IGF1R). Nucleic Acids Res 1992;20:1427.
38. Poduslo SE, Dean M, Kolch U, O'Brien SJ. Detecting high-resolution polymorphisms in human coding loci by combining PCR and single-strand conformation polymorphism (SSCP) analysis. Am J Hum Genet 1991;49:106–11.
39. Rudolph RE, Vaughan TL, Kristal AR, et al. Serum selenium levels in relation to markers of neoplastic progression among persons with Barrett's esophagus. JNCI Cancer Spectrum 2003;95:750–7.
40. Vaughan TL, Kristal AR, Blount PL, et al. Nonsteroidal anti-inflammatory drug use, body mass index, and anthropometry in relation to genetic and flow cytometric abnormalities in Barrett's esophagus. Cancer Epidemiol Biomarkers Prev 2002;11:745–52.
41. Reid BJ, Blount PL, Rubin CE, et al. Flow-cytometric and histological progression to malignancy in Barrett's esophagus: prospective endoscopic surveillance of a cohort. Gastroenterology 1992;102: 1212–9.
42. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 1988;16:1215.
43. Deal C, Ma J, Wilkin F, et al. Novel promoter polymorphism in insulin-like growth factor-binding protein-3: correlation with serum levels and interaction with known regulators. J Clin Endocrinol Metab 2001;86:1274–80.
44. Chiano MN, Clayton DG. Genotypic relative risks under ordered restriction. Genet Epidemiol 1998;15:135–46.
45. Chen SC, Chou CK, Wong FH, Chang CM, Hu CP. Overexpression of epidermal growth factor and insulin-like growth factor-I receptors and autocrine stimulation in human esophageal carcinoma cells. Cancer Res 1991;51:1898–903.
46. Liu YC, Leu CM, Wong FH, et al. Autocrine stimulation by insulin-like growth factor I is involved in the growth, tumorigenicity and chemoresistance of human esophageal carcinoma cells. J Biomed Sci 2002;9:665–74.
47. Takaoka M, Harada H, Andl CD, et al. Epidermal growth factor receptor regulates aberrant expression of insulin-like growth factor-binding protein 3. Cancer Res 2004;64:7711–23.
48. Jernstrom H, Barrett-Connor E. Obesity, weight change, fasting insulin, proinsulin, C-peptide, and insulin-like growth factor-1 levels

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in women with and without breast cancer: the Rancho Bernardo Study. J Womens Health Gend Based Med 1999;8:1265–72.
49. Giovannucci E, Haiman CA, Platz EA, et al. Dinucleotide repeat in the insulin-like growth factor-I gene is not related to risk of colorectal adenoma. Cancer Epidemiol Biomarkers Prev 2002;11:1509–10.
50. Schildkraut JM, Demark-Wahnefried W, Wenham RM, et al. IGF1 (CA)19 repeat and IGFBP-3 -202 A/C genotypes and the risk of prostate cancer in Black and White men. Cancer Epidemiol Biomarkers Prev 2005;14:403–8.
51. Allen NE, Davey GK, Key TJ, Zhang S, Narod SA. Serum insulin-like growth factor I (IGF-I) concentration in men is not associated with the cytosine-adenosine repeat polymorphism of the IGF-I gene. Cancer Epidemiol Biomarkers Prev 2002;11:319–20.
52. Kato I, Eastham J, Li B, Smith M, Yu H. Genotype-phenotype analysis for the polymorphic CA repeat in the insulin-like growth factor-I (IGF-I) gene. Eur J Epidemiol 2003;18:203–9.
53. Vinayek R, Pichney LS, Tantry U, et al. Characterization of insulin-like growth factor I receptors in human esophageal epithelial cells. Am J Physiol 1994;267:G105–14.
54. Peters G, Gongoll S, Langner C, et al. IGF-1R, IGF-1 and IGF-2 expression as potential prognostic and predictive markers in colorectal-cancer. Virchows Arch 2003;443:139–45.
55. Schips L, Zigeuner R, Ratschek M, et al. Analysis of insulin-like growth factors and insulin-like growth factor I receptor expression in renal cell carcinoma. Am J Clin Pathol 2004;122:931–7.
56. Cardillo MR, Monti S, Di Silverio F, et al. Insulin-like growth factor (IGF)-I, IGF-II and IGF type I receptor (IGFR-I) expression in prostatic cancer. Anticancer Res 2003;23:3825–35.
57. Abuzzahab MJ, Schneider A, Goddard A, et al. IGF-I receptor mutations resulting in intrauterine and postnatal growth retardation. N Engl J Med 2003;349:2211–22.
58. Renehan AG, Frystyk J, Flyvbjerg A. Obesity and cancer risk: the role of the insulin-IGF axis. Trends Endocrinol Metab 2006;17:328–36.
59. Ouban A, Muraca P, Yeatman T, Coppola D. Expression and distribution of insulin-like growth factor-1 receptor in human carcinomas. Human Pathology 2003;34:803–8.
60. Krajcik RA, Borofsky ND, Massardo S, Orentreich N. Insulin-like growth factor I (IGF-I), IGF-binding proteins, and breast cancer. Cancer Epidemiol Biomarkers Prev 2002;11:1566–73.
61. Goodman-Gruen D, Barrett-Connor E. Epidemiology of insulin-like growth factor-I in elderly men and women. The Rancho Bernardo Study. Am J Epidemiol 1997;145:970–6.
62. Ito Y, Nakachi K, Imai K, et al. Stability of frozen serum levels of insulin-like growth factor-I, insulin-like growth factor-II, insulin-like growth factor binding protein-3, transforming growth factor β, soluble Fas, and superoxide dismutase activity for the JACC study. J Epidemiol 2005;15 Suppl 1:S67–73.
63. Borofsky ND, Vogelman JH, Krajcik RA, Orentreich N. Utility of insulin-like growth factor-1 as a biomarker in epidemiologic studies. Clin Chem 2002;48:2248–51.

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Longitudinal Study of Insulin-like Growth Factor, Insulin-like Growth Factor Binding Protein-3, and their Polymorphisms: Risk of Neoplastic Progression in Barrett's Esophagus

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