Breast Cancer Res Treat (2011) 130:217–226
DOI 10.1007/s10549-011-1552-9

EPIDEMIOLOGY

Genotypes and phenotypes of IGF-I and IGFBP-3 in breast tumors among Chinese women

Biyun Qian · Hong Zheng · Herbert Yu · Kexin Chen

Received: 3 December 2010 / Accepted: 25 April 2011 / Published online: 12 May 2011

Abstract The relationship between IGF genotypes and phenotypes in breast tumors and their associations with breast cancer risk remain to be elucidated. Such information is especially scarce in Chinese women. To evaluate IGF-I and IGFBP-3 genotypes in relation to their phenotypes in local breast tissues and in association with breast cancer risk, we conducted a case–control study among Chinese women. The study recruited 403 breast cancer patients and 403 age-matched controls. Four single nucleotide polymorphisms (SNP) in the IGF-I gene (rs1520220, rs2946834, rs2195239, and rs7965399) and two SNPs of the IGFBP-3 gene (rs2854746 and rs2960436) with known correlations with their phenotypes in the circulation were genotyped using TaqMan assays. Fresh tumor samples from the same patients were analyzed with immunoassays for protein concentrations of IGF-I and IGFBP-3. Associations of breast cancer with these SNPs were examined using unconditional logistic regression. Correlations between IGF genotypes and phenotypes were determined with Wilcoxon rank-sum test. Of the six selected SNPs, only one IGF-I SNP (rs7965399) was associated with breast cancer risk in a recessive model (OR = 1.86; 95% CI: 1.04–3.32), and the association was more evident in patients who had menopause under age 50 or ER negative tumors. No associations were found between breast cancer and other three IGF-I and two IGFBP-3 SNPs. Patients with variant IGF-I or wild IGFBP-3 genotypes had higher peptide levels of IGF-I compared to those with wild IGF-I or variant IGFBP-3 genotypes. The selected IGF-I and IGFBP-3 SNPs did not show any strong evidence for being associated with breast cancer risk, but the genotypes were correlated with IGF-I phenotypes in tumor samples, suggesting possible influences of these SNPs on IGF-I activity in local tissues.

Keywords Breast cancer · IGF-I · IGFBP-3 · Genotype · Phenotype

B. Qian (✉) · H. Zheng · K. Chen
Department of Epidemiology and Biostatistics, Tianjin Medical University Cancer Institute and Hospital, Huan Hu Xi Rd, He Xi District, Tianjin 300060, People's Republic of China
e-mail: qianbiyun@yahoo.com.cn

B. Qian · H. Zheng · K. Chen
Key Laboratory of Breast Cancer Prevention and Therapy, Tianjin Medical University, Ministry of Education, Tianjin, People's Republic of China

H. Yu
Department of Epidemiology and Public Health, Yale Cancer Center, Yale University School of Medicine, New Haven, CT 06520-8034, USA

Introduction

Insulin-like growth factor-I (IGF-I) and insulin-like growth factor binding protein-3 (IGFBP-3) are important growth modulators [1]. In vitro studies demonstrate that IGF-I has strong mitogenic and anti-apoptotic effects on various cancer cells, including breast cancer [2–4]. Animal experiments suggest that IGF-I may promote tumorigenesis in the mammary gland [5, 6]. Several epidemiological studies show a positive correlation between circulating levels of IGF-I and breast cancer risk [7–13]. Genetic polymorphisms in the IGF-I and IGFBP-3 genes are also evaluated for their associations with breast cancer risk. The investigation initially focused on a limited number of genetic variations located mainly in the gene promoters or coding regions [14–17]. Recently, high-throughput analyses were used to interrogate the entire genes and their surrounding

regions. A large collaborative study, the Breast and Prostate Cancer Cohort Consortium (BPC3), recently reported important influences of several single nucleotide polymorphisms (SNP) in the IGF-I and IGFBP-3 genes on circulating levels of the IGF-I and IGFBP-3, although no association was found with breast cancer risk [18].

Despite a large body of evidence indicating the possible involvement of IGF-I in breast cancer, a causal link between the growth factor and breast cancer remains to be established [15, 18–24]. Furthermore, most of the human studies are conducted among Caucasians; very few have focused on other races and ethnicities. The relationship between IGF genotype and phenotype is also investigated mainly in Caucasians; data from other racial groups are scarce. To further elucidate the role of IGF-I in breast cancer, we conducted a case–control study of breast cancer in Chinese women. In the study, we analyzed the associations of breast cancer risk with four IGF-I and two IGFBP-3 SNPs which were known to have influences on their phenotypes in the circulation, and examined the correlation between the genotypes of IGF-I and IGFBP-3 and their phenotypes in breast tumor tissues.

Methods

Patients and controls

Patients who underwent surgery at Tianjin Medical University Cancer Hospital between January 2007 and December 2007 with newly diagnosed and histologically confirmed primary breast cancer were recruited for this study. Clinical information collected for the study included histology, tumor size, lymph node involvement, disease stage, and status of estrogen receptor and progesterone receptor. Genetically unrelated control subjects with frequency-match to their cases by age (±5 years) were selected from women living in the neighboring communities where the patients resided.

The study was approved by an ethical review committee at Tianjin Medical University Cancer Hospital. All of the study participants signed an informed consent and completed a structured questionnaire which elicited information on demographic features and several risk factors of breast cancer including age at diagnosis or interview, age at menarche, age at menopause, smoking status, physical activity, numbers of pregnancy and live birth, breast feeding, body mass index, use of oral contraceptives, a history of benign breast diseases, and a family history of breast cancer in the first-degree relatives. Individuals who smoked at least one cigarette per day for half a year were considered smokers. For physical activity, less than one time or 1 h per week of any moderate–to-vigorous-intensity aerobic activity in the past 6 months was defined as "never"; otherwise it was "ever." A blood sample (10 ml) was collected from each study subject, and buffy coats were isolated from the blood for DNA extraction and genotyping. Tumor samples from the patients were also obtained from a tissue bank at Tianjin Medical University Cancer Hospital, which routinely collects and stores tumor specimens from the operated patients upon approval by the Institution Review Board at Tianjin Medical University Cancer Hospital.

Analysis of IGF genotypes

Genomic DNA was extracted from buffy coats using the QIAGEN DNA Blood Mini Kit (QIAGEN Inc., Valencia, CA), and the extraction was performed according to the manufacturer's instructions. SNPs with possible influences on their phenotypes in the circulation reported by a recent GWAS [18] were selected for investigation, which included four in the IGF-I gene (rs1520220, rs2195239, rs2946834, and rs7965399) and two in the IGFBP-3 gene (rs2854746 and rs2960436).

All of the chosen SNPs were genotyped with the TaqMan assays which were purchased from ABI (Applied Biosystems, Foster City, CA). In each genotyping assay, 10 ng genomic DNA was mixed with a PCR cocktail that contained two fluorescence-labeled allele specific probes (200 nM each), forward and reverse primers (1 μM each), and 5 μl of 2 × TaqMan Universal PCR Master Mix including ROX passive dye as internal control, dNTPs, PCR buffer, and *Taq* polymerase (Applied Biosystems, Foster City, CA) in a final volume of 10 μl. PCR amplification was achieved under the condition of one cycle of denaturing at 95°C for 10 min and 45 cycles of denaturing at 92°C for 15 s and annealing and elongation at 60°C for 1 min, followed by storage at 4°C. The fluorescence of PCR products was then plotted, and the genotypes were determined according to the allelic discrimination software. Water control and previously genotyped samples were included in each plate to monitor genotyping quality. Five percent of the samples were randomly selected for retesting, and the results of repeated tests were in complete concordance. All TaqMan assays were performed with the ABI 7500 thermal cycler (Applied Biosystems, Foster City, CA).

Analysis of IGF phenotypes

Freshly frozen tumor specimens were processed to extract tissue proteins for analysis of IGF-I and IGFBP-3. Tumor tissues were first pulverized in liquid nitrogen and the tissue powders (~100 mg) were mixed with 1 ml BD TALON × Tractor buffer (Clontech, Palo Alto, CA) which

was centrifuged at 14,000 rpm for 30 min at 4°C. After centrifugation, the supernatants were collected for measurement of total proteins, IGF-I and IGFBP-3. Concentrations of total proteins were determined using the bicinchoninic acid method (Pierce Inc., Rockford, IL). Levels of IGF-I and IGFBP-3 peptides were measured with enzyme-linked immunosorbent assays (ELISA) (Diagnostic Laboratories Systems, Webster, TX) following the manufacturer's protocols, respectively. The tumor samples were tested in duplicate, and the test results with CV greater than 10% were repeated. IGF peptide concentrations in tumor samples were adjusted for total proteins.

Statistical analysis

Chi-square test was used to compare the distributions of demographic variables, known breast cancer risk factors, and IGF genotypes between the cases and controls. Analysis of the Hardy–Weinberg equilibrium for each SNP was also performed among the control subjects. Unconditional logistic regression models were developed to examine the associations between SNPs and breast cancer risk. In the logistic regression analysis, odds ratios (ORs) and their 95% confidence intervals (95% CI) were calculated without and with adjustment for other risk factors and confounding variables, including age at diagnosis or interview, number of pregnancy, a history of benign breast diseases, and a family history of breast cancer. The associations between the IGF genotypes and breast cancer risk were also analyzed after the study subjects were stratified by menopausal status, age at menopause, BMI, family history of breast cancer in the first-degree of relatives, age at menarche, use of oral contraceptives, as well as clinical and pathological features of breast cancer, including histology, tumor size, lymph node involvement, disease stage, and ER and PR statuses. Based on the molecular weight of IGF-I and IGFBP-3, a molar ratio between IGF-I and IGFBP-3 was calculated. Wilcoxon rank-sum test was used to compare levels of IGF-I and IGFBP-3 among different IGF genotypes. SAS (SAS, Cary, and NC) was used for all the analyses. In addition, pairwise linkage disequilibrium (LD) values were calculated using the expectation–maximization (EM) algorithm. Haplotype frequencies were estimated using the SAS Haplotype program and SHESIS software (http://analysis.bio-x.cn) [25].

Results

Patient characteristics

Baseline characteristics of the patients and controls are presented in Table 1. As expected, the patients reported higher percentages of known breast cancer risk factors than the controls, including fewer numbers of pregnancies and less physical activity. Breast cancer patients were also more likely than the controls to have a history of benign breast diseases or a family history of breast cancer in the first-degree relatives. Clinical and pathological features of breast cancer are also shown in Table 1. Three hundred and twenty patients (80%) had infiltrating ductal carcinoma (IDC), and 60 patients (15.4%) were diagnosed with stage I disease. Using 10% as cutoff for hormone receptor status, 213 tumors (53.5%) were estrogen receptor (ER) positive, and 234 (58.8%) were progesterone receptor (PR) positive.

IGF genotypes and breast cancer risk

Four SNPs in the IGF-I genes and two in the IGFBP-3 gene were examined in the study. All the SNPs investigated in the study were in Hardy–Weinberg equilibrium among the control subjects. Table 2 shows the genotype frequency of each polymorphism in the cases and controls, along with the corresponding ORs after adjusting for age, frequency of pregnancy, a history of benign breast diseases, and a family history of breast cancer. Three genetic models, including co-dominant, dominant, and recessive, were utilized to calculate the ORs. Of the four SNPs examined in the IGF-I gene, only one (rs7965399) was shown to have a significant association with breast cancer in a recessive model. Compared to those with the 'T' allele (wild genotype), either one or two, women with homozygous 'CC' genotype (variant genotype) had increased risk of breast cancer (OR = 1.86; 95% CI: 1.04–3.32). Subgroup analyses stratified by age at menopause, family history of breast cancer, histology, or ER status were also performed for this SNP. In the stratified analyses, we found that the risk association with rs7965399 was more evident among those who had menopause at younger ages. This polymorphism also showed stronger associations in patients with IDC (OR = 2.18; 95% CI: 1.08–4.38) and ER-negative tumors (OR = 2.48; 95% CI: 1.08–5.67), but not in those with ER-positive tumors (Table 3). None of the IGFBP-3 polymorphisms were found to be associated with breast cancer risk.

Next, we evaluated the pairwise LD and haplotype distribution among these SNPs. The results indicated that three of the four IGF-I SNPs (rs1520220, rs2946834, and rs2195239) as well as two of the IGFBP-3 SNPs were in LD, and the lowest D′ was greater than 0.90; r^2 greater than 0.72 (data not shown). The most common haplotypes in the IGF-I gene were "CCG" and "GTC," which accounted for 91.2% of the cases and 92.9% of the controls; the most common haplotypes in the IGFBP-3 gene were "GA" and "CG," which accounted for 98.9% of the cases and 98.4% of the controls. The distributions of the common

Table 1 Distributions of risk factors between breast cancer cases and control subjects

Variables	No. of subjects (%)		OR (95% CI)	*P* value
	Case ($n = 403$)	Control ($n = 403$)		
Age (years)				
≤50	165 (40.9)	154 (38.2)	1.00	0.428
>50	238 (59.1)	249 (61.8)	0.89 (0.67–1.18)	
Age at menarche (years)				
≤12	42 (10.4)	45 (11.2%)	1.00	0.715
>12	361 (89.6)	356 (88.8%)	1.09 (0.70–1.70)	
BMI				
<25	216 (54.4)	215 (54.0)	1.00	0.973
25–30	145 (36.5)	145 (36.4)	1.00 (0.74–1.34)	
>30	36 (9.1)	38 (9.6)	0.94 (0.58–1.55)	
Number of pregnancy (≥5 months)				
0–1	224 (57.4)	120 (42.7)	1.00	**0.000**
≥2	166 (42.6)	161 (57.3)	**0.55 (0.41–0.75)**	
Number of live birth				
0–1	227 (58.2)	216 (55.2)	1.00	0.404
≥2	163 (41.8)	175 (44.8)	0.89 (0.67–1.18)	
Breast feeding				
No	46 (11.8)	44 (11.3)	1.00	0.823
Yes	344 (88.2)	346 (88.7)	0.95 (0.61–1.48)	
Time of breast feeding				
≤12	109 (33.3)	111 (33.1)	1.00	0.957
>12	218 (66.7)	224 (66.9)	0.99 (0.72–1.37)	
Oral contraceptive use				
Never	304 (82.6)	334 (85.6)	1.00	0.253
Ever	64 (17.4)	56 (14.4)	1.26 (0.85–1.86)	
Natural menopause				
No	168 (42.4)	156 (39.2)	1.00	0.548
Yes	228 (57.6)	231(59.7)	0.92 (0.69–1.22)	
Benign breast disease				
Never	306 (76.1)	353 (89.6)	1.00	**<0.001**
Ever	96 (23.9)	41 (10.4)	2.70 (1.82–4.02)	
Family history of breast cancer				
No	277 (68.9)	322 (80.7)	1.00	**0.000**
Yes	125 (31.1)	77 (19.3)	1.89 (1.36–2.62)	
Smoking status				
Never	328 (87.5)	356 (90.6)	1.00	0.166
Ever	47 (12.5)	37 (9.4)	1.38 (0.87–2.18)	
Physical exercise				
Never	238 (63.5)	116 (49.8)	1.00	**0.001**
Ever	137 (36.5)	117 (50.2)	**0.57 (0.41–0.80)**	

Clinical features	Frequency	Proportion (%)
Stage		
0–I	60	15.5
II	243	62.8
III + IV	84	21.7
Histology		
IDC[a]	320	80.0
Others	80	20.0
Tumor size		
≤2 cm	91	23.3
>2 cm	300	76.7
Node		
Negative	209	53.2
Positive	184	46.8
ER[b]		
Negative	185	46.5
Positive	213	53.5
PR[c]		
Negative	164	41.2
Positive	234	58.8

Bold values indicate $P < 0.05$

[a] Infiltrating ductal carcinoma

[b] Estrogen receptor

[c] Progesterone receptor

haplotypes were not significantly different between the cases and controls, either in the IGF-I or IGFBP-3 gene ($P = 0.290$ and 0.445, respectively).

IGF genotype and phenotype correlation

To assess the genotype and phenotype correlation, protein concentrations of IGF-I and IGFBP-3 in breast tumor samples were analyzed with ELISAs. Our analyses suggested that peptide levels of IGF-I were inversely correlated with age and menopause status (data not shown). The results also indicated that tumor levels of IGF-I were higher in women with variant alleles of IGF-I SNPs than those with the homozygous wild genotypes. For IGFBP-3 SNPs, however, the homozygous variant genotypes had lower IGF-I compared to their wild counterparts. The differences in phenotype, either in IGF-I alone or the molar ratio of IGF-I to IGFBP-3, were statistically significant or borderline significant in two IGF-I intron SNPs (rs1520220 and rs2195239) and two IGFBP-3 SNPs (rs2854746 and rs2960436) (Table 4). No significant correlation was found between IGFBP-3 genotype and phenotype in the tissue (data not shown).

Discussion

In this study, we selected four IGF-I and two IGFBP-3 SNPs, which are known to be associated with their phenotypes in the circulation among Caucasians, to investigate their

Table 2 Associations of breast cancer risk with IGF-I and IGFBP-3 polymorphisms

Genotype	Case (%) $N = 403$	Control (%) $N = 403$	P value	OR[a] (95% CI)	OR[a] (95% CI) ② + ③ vs. ①	OR[a] (95% CI) ③ vs.① + ②
IGF-I (rs1520220)						
CC	143 (35.5)	132 (32.8)		1.00	1.00	1.00
CG	189 (46.9)	193 (47.9)	0.668	0.90 (0.65–1.25)	0.89 (0.66–1.21)	0.93 (0.64–1.34)
GG	71 (17.6)	78 (19.4)		0.87 (0.58–1.32)		
IGF-I (rs2946834)						
CC	120 (29.8)	118 (29.3)		1.00	1.00	1.00
CT	210 (52.1)	204 (50.6)	0.771	1.06 (0.76–1.48)	1.03 (0.75–1.41)	0.90 (0.62–1.30)
TT	73 (18.1)	81 (20.1)		0.94 (0.61–1.43)		
IGF-I (rs2195239)						
GG	147 (36.5)	135 (33.5)		1.00	1.00	1.00
CG	181 (44.9)	193 (47.9)	0.639	0.83 (0.60–1.15)	0.86 (0.64–1.17)	1.06 (0.73–1.54)
CC	75 (18.6)	75 (18.6)		0.95 (0.63–1.44)		
IGF-I (rs7965399)						
TT	212 (52.6)	211 (52.4)		1.00	1.00	1.00
CT	156 (38.7)	170 (42.2)	0.168	0.88 (0.65–1.19)	0.98 (0.73–1.30)	**1.86 (1.04–3.32)**
CC	35 (8.7)	22 (5.5)		1.76 (0.97–3.19)		
IGFBP-3 (rs2854746)						
GG	219 (54.3)	229 (56.8)		1.00	1.00	1.00
CG	163 (40.5)	155 (38.5)	0.769	1.06 (0.79–1.44)	1.06 (0.80–1.42)	1.04 (0.53–2.04)
CC	21 (5.2)	19 (4.7)		1.07 (0.54–2.11)		
IGFBP-3 (rs2960436)						
AA	220 (54.6)	231 (57.3)		1.00	1.00	1.00
AG	162 (40.2)	152 (37.7)	0.737	1.09 (0.81–1.47)	1.08 (0.81–1.44)	0.97 (0.50–1.88)
GG	21 (5.2)	20 (5.0)		1.00 (0.51–1.97)		

Bold values indicate $P < 0.05$

[a] Adjusted by age, number of pregnancy, benign breast diseases, and a family history of breast cancer

① Homozygous wild genotype, ② heterozygous genotype, ③ homozygous variant genotype

associations with breast cancer risk among Chinese women. Table 5 shows the allele frequencies of the six selected SNPs by ancestry recorded by the NCBI dbSNP database as well as research findings reported by recent studies of these SNPs in association with cancer risk. The allele frequencies of these SNPs in our study were similar to those of Asians, but different from those of Europeans in the database. The IGF-I SNP rs7965399 seems to be quite different between Asians and Caucasians. The frequency of the variant allele is quite high among Asians, but virtually none in Caucasians. This racial difference may indicate the possibility that an association between the SNP rs7965399 and a disease differs by race. This possibility seems to exist when we compare our finding with other studies on SNP rs7965399. IGF-I rs7965399 was found to be associated with breast cancer risk among Chinese women in our study, and the association seemed to be more evident in women with early age at menopause, or having ER negative tumors, but the association was not seen in Caucasian women. However, other SNPs investigated showed no association with breast cancer risk, which was consistent with the finding in Caucasian women. Interestingly, most of the SNPs investigated in this study also showed a possible impact on IGF-I phenotype in breast tumors. These results seemed to be consistent with the finding of Caucasian women when blood samples were used for evaluation of phenotype.

IGF-I rs7965399 was the only SNP that was found to be associated with breast cancer risk in our study. This association, however, was shown only in a recessive model; no relationship was indicated in other genetic models. Besides our study, there was another investigation which found the same SNP to be associated with prostate cancer risk [26]. Both that and our studies indicated that the individuals with variant rs7965399 had elevated cancer risk. The SNP is located in the 5′-noncoding region of the IGF-I gene, which is near the transcription site. Based on the location, it is plausible that SNP rs7965399 may have a potential influence on the regulation of IGF-I activity through its impact on IGF-I transcription. In addition, it is also possible that this polymorphism may be associated with breast cancer

Table 3 Associations of breast cancer risk with IGF-I (rs7965399) polymorphism stratified by selected variables

Variable	Cases	Controls	*P* value	OR (95% CI)	OR[a] (95% CI)
Age of menopause (<50 year)					
TT	46 (47.9%)	62 (50.8%)	0.220	1.00	1.00
CT	37 (38.5%)	52 (42.6%)		1.17 (0.60–2.27)	1.20 (0.59–2.45)
CC	13 (13.5%)	8 (6.6%)		**3.86 (1.20–12.39)**	**5.07 (1.43–18.03)**
Age of menopause (≥50 year)					
TT	80 (60.2%)	62 (52.1%)	0.346	1.00	1.00
CT	43 (32.3%)	49 (41.2%)		0.70 (0.39–1.25)	0.62 (0.34–1.15)
CC	10 (7.5%)	8 (6.7%)		1.07 (0.36–3.23)	1.10 (0.34–3.57)
Family history of breast cancer (Never)					
TT	144 (52.0%)	176 (54.7%)	0.571	1.00	1.00
CT	111 (40.1%)	127 (39.4%)		1.21 (0.83–1.76)	1.20 (0.81–1.78)
CC	22 (7.9%)	19 (5.9%)		1.54 (0.74–3.20)	1.47 (0.69–3.13)
Family history of breast cancer (ever)					
TT	68 (54.4%)	34 (44.2%)	**0.014**	1.00	1.00
CT	44 (35.2%)	41 (53.3%)		0.54 (0.27–1.08)	0.57 (0.28–1.17)
CC	13 (10.4%)	2 (2.6%)		5.32 (0.91–31.03)	**11.29 (1.15–110.94)**
Tumor Size (<2 cm)					
TT	46 (50.6%)	211 (52.4%)	0.028	1.00	1.00
CT	33 (36.3%)	170 (42.2%)		0.81 (0.47–1.38)	0.73 (0.42–1.29)
CC	12 (13.2%)	22 (5.5%)		**2.58 (1.02–6.57)**	**2.90 (1.11–7.57)**
Tumor Size (≥2 cm)					
TT	158 (52.7%)	211 (52.4%)	0.454		
CT	119 (39.7%)	170 (42.2%)		1.10 (0.77–1.55)	1.11 (0.77–1.60)
CC	23 (7.7%)	22 (5.5%)		1.65 (0.83–3.30)	1.70 (0.82–3.54)
Histology (Infiltrating duct carcinoma)					
TT	164 (51.3%)	211 (52.4%)	0.179	1.00	1.00
CT	127 (39.7%)	170 (42.2%)		1.04 (0.74–1.47)	1.01 (0.71–1.45)
CC	29 (9.1%)	22 (5.5%)		**1.98 (1.02–3.85)**	**2.18 (1.08–4.38)**
Histology (others)					
TT	47 (58.8%)	211 (52.4%)	0.440	1.00	1.00
CT	27 (33.8%)	170 (42.2%)		0.88 (0.50–1.56)	0.91 (0.50–1.65)
CC	6 (7.5%)	22 (5.5%)		1.19 (0.39–3.59)	1.27 (0.39–4.12)
ER (Negative)					
TT	100 (54.1%)	211 (52.4%)	0.118	1.00	1.00
CT	67 (36.2%)	170 (42.2%)		0.94 (0.62–1.40)	0.94 (0.62–1.44)
CC	18 (9.7%)	22 (5.5%)		**2.23 (1.01–4.90)**	**2.48 (1.08–5.67)**
ER (Positive)					
TT	109 (51.2%)	211 (52.4%)	0.517	1.00	1.00
CT	87 (40.9%)	170 (42.2%)		1.03 (0.70–1.51)	0.96 (0.64–1.44)
CC	17 (8.0%)	22 (5.5%)		1.41 (0.67–2.98)	1.60 (0.73–3.50)

Bold values indicate $P < 0.05$

[a] Adjusted by age, benign breast diseases, number of pregnancy, a family history of breast cancer, and other SNPs

risk through its LD with a functional SNP in the IGF-I gene. Further evaluation of the functional relevance of IGF-I rs7965399 appears to be warranted.

Other IGF-I SNPs investigated in this study (rs1520220, rs2946834, and rs2195239) are located in different regions of the IGF-I gene, including introns and 3' UTR. So far, only one study has reported an association between rs1520220 and breast cancer risk [20]. Although two recent studies indicated possible associations of rs1520220 and rs2946834 with breast mammographic density, which could be indirectly linked to breast cancer risk [27, 28], most genotype studies failed to demonstrate any associations between

Table 4 Associations of IGF-I and IGFBP3 genotypes and phenotypes in breast tumors

Genotype	IGF-I			Molar Ratio of IGF-I to IGFBP-3	
	N	Median (range)	*P* value	Median (range)	*P* value
IGF-I (rs1520220)					
CC	143	111.22 (7.80–412.63)	0.079	27.10 (2.39–112.20)	0.165
CG	189	141.41 (14.63–444.02)		32.48 (4.46–115.85)	
GG	71	121.82 (18.07–328.43)		30.87 (3.53–186.37)	
CC	143	111.22 (7.80–412.63)	**0.047**	27.10 (2.39–112.20)	0.058
CG/GG	260	135.42 (16.97–438.83)		31.88 (4.18–135.83)	
IGF-I (rs2946834)					
CC	120	115.05 (8.49–422.45)	0.386	27.91 (1.94–108.04)	0.448
CT	210	136.01 (14.47–433.64)		31.27 (3.91–125.38)	
TT	73	121.82 (18.07–328.43)		29.97 (3.53–186.37)	
CC	120	115.05 (8.49–422.45)	0.270	27.91 (1.94–108.04)	0.205
CT/TT	283	129.81 (16.58–411.18)		30.87 (3.91–136.70)	
IGF-I (rs2195239)					
GG	147	112.30 (7.80–371.75)	0.129	26.32 (2.39–112.20)	0.064
CG	181	137.91 (14.63–444.02)		33.32 (4.46–121.91)	
CC	75	129.79 (19.01–328.43)		30.87 (3.53–143.74)	
GG	147	112.30 (7.81–371.75)	**0.043**	26.32 (2.39–112.20)	**0.020**
CG/CC	256	134.76 (16.58–444.02)		32.72 (3.91–136.70)	
IGF-I (rs7965399)					
TT	212	118.39 (7.81–444.02)	0.201	28.39 (2.71–115.85)	0.228
CT	156	132.41 (19.01–412.63)		33.00 (5.83–147.66)	
CC	35	148.11 (14.63–313.67)		28.40 (2.62–140.26)	
TT	212	118.39 (7.81–444.02)	0.077	28.39 (2.71–115.85)	0.095
CT/CC	191	134.12 (18.58–389.64)		30.73 (4.46–143.74)	
IGFBP-3 (rs2854746)					
GG	219	127.07 (10.80–431.90)	0.127	29.02 (3.75–143.74)	**0.042**
CG	163	135.44 (15.66–427.53)		33.52 (3.57–121.91)	
CC	21	73.29 (24.11–260.75)		16.78 (2.39–64.01)	
GG/CG	382	129.81 (12.43–427.53)	**0.047**	30.77 (3.75–134.96)	**0.015**
CC	21	73.29 (24.11–260.75)		16.78 (2.39–64.01)	
IGFBP-3 (rs2960436)					
AA	220	129.81 (11.00–424.63)	0.148	29.22 (3.79–143.05)	0.083
AG	162	129.28 (15.66–427.53)		33.86 (3.57–121.91)	
GG	21	73.29 (24.11–260.75)		18.48 (2.39–64.01)	
AA/AG	382	129.81 (12.43–427.53)	0.054	30.59 (3.75–134.96)	**0.031**
GG	21	73.29 (24.11–260.75)		18.48 (2.39–64.01)	

Bold values indicate $P < 0.05$

genetic polymorphisms in the IGF-I gene and breast cancer risk [18, 21, 22]. For the polymorphisms in IGFBP-3, one SNP (rs2854744, A-202C) in the promoter region has been studied extensively, but its association with cancer risk remains to be inconclusive. Other IGFBP-3 SNPs have not been found to be associated with breast cancer risk [13, 16, 22, 28–30]. A large consortium study of 6,912 breast cancer cases and 8,891 matched controls also found no associations between breast cancer risk and SNPs in the IGF-I and IGFBP-3 genes among Caucasian women [18]. Moreover, a recent meta-analysis of 96 studies concluded no apparent influences of several well-studied polymorphisms in the IGF-I and IGFBP-3 genes on breast cancer risk [13]. Similar to these results, we found no associations between breast cancer risk and the IGF-I and IGFBP-3 SNPs among Chinese women, either as a single SNP or in their haplotypes.

Numerous studies have shown that high IGF-I and low IGFBP-3 in the circulation are associated with increased risk

Table 5 Allele frequencies of IGF-1, IGFBP3 genetic polymorphisms, and the studies of these SNPs in association with cancer risk

No	Gene	Location	Allele Frequencies (Minor/Major)[a]		Study Author	Tumor type	OR (95% CI)]
			European	HCB/Asian			
	IGF-I	Chromosome 12					
1	rs1520220	Intron 3	0/0.759	0.111/0.378	Al-Zahrani [20]	Breast	1.41 (1.11–1.79)
					Cheng [26]	Prostate	No
					Tamimi [27]	Breast	N/A
					Diorio [28]	Breast	N/A
					Patel [18]	Breast	No
2	rs2946834	3′ UTR	0.097/0.540	0.140/0.326	Cheng 26]	Prostate	No
					Tamimi [27]	Breast	N/A
					Patel [18]	Breast	No
3	rs2195239	Intron 2	0.067/0.650	0.133/0.400	Cheng [26]	Prostate	No
					Patel [18]	Breast	No
4	rs7965399	5′ UTR	0/0.956	0.047/0.488	Cheng [26]	Prostate	1.26 (0.95–1.68)
					Hernandez [33]	Prostate	0.8 (0.3 –2.1)
					Patel [18]	Breast	No
	IGFBP-3	Chromosome 7					
1	rs2854746 (Ala32Gly)	Exon 1	0.455/0.227	0.286/0.429	Pechlivanis [34]	Colorectum	No
					Feik [35]	Colorectum	No
					Terry [36]	Ovary	No
					Patel [18]	Breast	No
2	rs2960436	Intron	0.364/0.227	0.167/0.500	Patel [18]	Breast	No

[a] Allele frequencies are from NCBI SNP database

of breast cancer, but these findings are challenged by the speculation that circulating IGF-I and IGFBP-3 may differ from those in local tissues either in quantity or quality. IGF-I and IGFBP-3 in breast tissues are derived from both local expression and systemic regulation [31]. Recent studies indicated that IGF-I polymorphisms were associated with breast mammographic density, especially the SNP rs1520220 which was reported by two independent studies to be strongly associated with breast mammographic density [27, 28]. This SNP is located in the third intron with unclear biological implications. Our study showed that this SNP was associated with the phenotype of IGF-I in breast tissue. Another intron SNP rs2195239, which was in LD with this one, was also shown to be significantly associated with the IGF phenotype in breast tissue. Moreover, two IGFBP-3 polymorphisms investigated were correlated with the phenotype of IGF-I, both in IGF-I peptide levels and the molar ratio of IGF-I to IGFBP-3. The latter reflects the bioavailability of IGF-I in local tissues. The correlation between IGFBP-3 genotype and local phenotype, however, was not observed in our study. Our finding seems to be different from the results of two previous studies which analyzed IGFBP-3 phenotype in the circulation [18, 28]. Two clinical studies found that IGFBP-3 polymorphisms or IGFBP-3 mRNA expression, but not its protein concentrations, had some influences on breast cancer survival [31, 32].

IGFBP-3 binds to approximately 90% of IGF-I in the circulation. This binding inhibits IGF-I interacting with its receptor IGF-IR, and reduces the IGF signal or activity [22]. Based on these understandings, we speculate that our findings of correlations between IGFBP-3 genotypes and IGF-I phenotypes in local tissues may be mediated through the effects of IGFBP-3 genotypes on its phenotype in the circulation. Our results also indicate that SNPs in the IGF-I and IGFBP-3 genes may affect levels of IGF-I expression not only in the circulation but also in local tissues, which provides new insights into the relationship of IGF genotype and phenotype.

To our knowledge, this is the first study that evaluates the relationship between IGF genotype and phenotype in breast tumors among Chinese women. Our finding suggests that genetic polymorphisms in the IGF-I and IGFBP-3 genes may affect IGF-I expression in local tissues. Although there were possible influences of IGF-I genotypes on its phenotype in breast tumors, we found no strong evidence that genetic polymorphisms in the IGF-I and IGFBP-3 genes were associated with breast cancer risk. One IGF-I SNP rs7965399, however, was shown to be associated with breast cancer risk, and this association seemed to be more evident in women with early menopause or having ER-negative tumors.

References

1. Moschos SJ, Mantzoros CS (2002) The role of the IGF system in cancer: from basic to clinical studies and clinical applications. Oncology 63(4):317–332
2. Wood TL, Yee D (2000) Introduction: IGFs and IGFBPs in the normal mammary gland and in breast cancer. J Mammary Gland Biol Neoplasia 5(1):1–5
3. Sachdev D, Yee D (2001) The IGF system and breast cancer. Endocr Relat Cancer 8(3):197–209
4. Marshman E, Streuli CH (2002) Insulin-like growth factors and insulin-like growth factor binding proteins in mammary gland function. Breast Cancer Res 4(6):231–239
5. Hadsell DL, Bonnette SG (2000) IGF and insulin action in the mammary gland: lessons from transgenic and knockout models. J Mammary Gland Biol Neoplasia 5(1):19–30
6. Laban C, Bustin SA, Jenkins PJ (2003) The GH-IGF-I axis and breast cancer. Trends Endocrinol Metab 14(1):28–34
7. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M (1998) Circulating concentrations of insulin-like growth factor-I and risk of breast cancer. Lancet 351(9113):1393–1396
8. Toniolo P, Bruning PF, Akhmedkhanov A, Bonfrer JM, Koenig KL, Lukanova A, Shore RE, Zeleniuch-Jacquotte A (2000) Serum insulin-like growth factor-I and breast cancer. Int J Cancer 88(5):828–832
9. Yu H, Jin F, Shu XO, Li BD, Dai Q, Cheng JR, Berkel HJ, Zheng W (2002) Insulin-like growth factors and breast cancer risk in Chinese women. Cancer Epidemiol Biomarkers Prev 11(8):705–712
10. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M (2004) Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet 363(9418):1346–1353
11. Allen NE, Roddam AW, Allen DS, Fentiman IS, Dos Santos Silva I, Peto J, Holly JM, Key TJ (2005) A prospective study of serum insulin-like growth factor-I (IGF-I), IGF-II, IGF-binding protein-3 and breast cancer risk. Br J Cancer 92(7): 1283–1287
12. Canzian F, McKay JD, Cleveland RJ, 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(10): 2316–2325
13. Chen W, Wang S, Tian T, Bai J, Hu Z, Xu Y, Dong J, Chen F, Wang X, Shen H (2009) Phenotypes and genotypes of insulin-like growth factor 1, IGF-binding protein-3 and cancer risk: evidence from 96 studies. Eur J Hum Genet 17(12):1668–1675
14. Yu H, Li BD, Smith M, Shi R, Berkel HJ, Kato I (2001) Polymorphic CA repeats in the IGF-I gene and breast cancer. Breast Cancer Res Treat 70(2):117–122
15. Setiawan VW, Cheng I, Stram DO, Penney KL, Le Marchand L, Altshuler D, Kolonel LN, Hirschhorn J, Henderson BE, Freedman ML (2006) Igf-I genetic variation and breast cancer: the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 15(1):172–174
16. Ren Z, Cai Q, Shu XO, Cai H, Li C, Yu H, Gao YT, Zheng W (2004) Genetic polymorphisms in the IGFBP3 gene: association with breast cancer risk and blood IGFBP-3 protein levels among Chinese women. Cancer Epidemiol Biomarkers Prev 13(8): 1290–1295
17. Qiu LX, Yao L, Yuan H, Mao C, Chen B, Zhan P, Xue K, Zhang J, Hu XC (2010) IGFBP3 A-202C polymorphism and breast cancer susceptibility: a meta-analysis involving 33,557 cases and 45,254 controls. Breast Cancer Res Treat 122(3):867–871
18. Patel AV, Cheng I, Canzian F, Le Marchand L, Thun MJ, Berg CD, Buring J, Calle EE, Chanock S, Clavel-Chapelon F et al (2008) IGF-1, IGFBP-1, and IGFBP-3 polymorphisms predict circulating IGF levels but not breast cancer risk: findings from the Breast and Prostate Cancer Cohort Consortium (BPC3). PLoS One 3(7):e2578
19. Rohrbacher M, Risch A, Kropp S, Chang-Claude J (2005) The A(-336C) insulin-like growth factor binding protein-3 promoter polymorphism is not a modulator of breast cancer risk in Caucasian women. Cancer Epidemiol Biomarkers Prev 14(1):289–290
20. Al-Zahrani A, Sandhu MS, Luben RN, Thompson D, Baynes C, Pooley KA, Luccarini C, Munday H, Perkins B, Smith P et al (2006) IGF1 and IGFBP3 tagging polymorphisms are associated with circulating levels of IGF1, IGFBP3 and risk of breast cancer. Hum Mol Genet 15(1):1–10
21. Cheng I, Penney KL, Stram DO, Le Marchand L, Giorgi E, Haiman CA, Kolonel LN, Pike M, Hirschhorn J, Henderson BE et al (2006) Haplotype-based association studies of IGFBP1 and IGFBP3 with prostate and breast cancer risk: the multiethnic cohort. Cancer Epidemiol Biomarkers Prev 15(10):1993–1997
22. Canzian F, McKay JD, Cleveland RJ, 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(2):299–307
23. Wagner K, Hemminki K, Forsti A (2007) The GH1/IGF-1 axis polymorphisms and their impact on breast cancer development. Breast Cancer Res Treat 104(3):233–248
24. Slattery ML, Sweeney C, Wolff R, Herrick J, Baumgartner K, Giuliano A, Byers T (2007) Genetic variation in IGF1, IGFBP3, IRS1, IRS2 and risk of breast cancer in women living in Southwestern United States. Breast Cancer Res Treat 104(2):197–209
25. Li Z, Zhang Z, He Z, Tang W, Li T, Zeng Z, He L, Shi Y (2009) A partition-ligation-combination-subdivision EM algorithm for haplotype inference with multiallelic markers: update of the SHEsis (http://analysis.bio-x.cn). Cell Res 19(4): 519–523
26. Cheng I, Stram DO, Penney KL, Pike M, Le Marchand L, Kolonel LN, Hirschhorn J, Altshuler D, Henderson BE, Freedman ML (2006) Common genetic variation in IGF1 and prostate cancer risk in the Multiethnic Cohort. J Natl Cancer Inst 98(2):123–134
27. Tamimi RM, Cox DG, Kraft P, Pollak MN, Haiman CA, Cheng I, Freedman ML, Hankinson SE, Hunter DJ, Colditz GA (2007) Common genetic variation in IGF1, IGFBP-1, and IGFBP-3 in relation to mammographic density: a cross-sectional study. Breast Cancer Res 9(1):R18
28. Diorio C, Brisson J, Berube S, Pollak M (2008) Genetic polymorphisms involved in insulin-like growth factor (IGF) pathway in relation to mammographic breast density and IGF levels. Cancer Epidemiol Biomarkers Prev 17(4):880–888
29. Schernhammer ES, Hankinson SE, Hunter DJ, Blouin MJ, Pollak MN (2003) Polymorphic variation at the -202 locus in IGFBP3: Influence on serum levels of insulin-like growth factors, interaction with plasma retinol and vitamin D and breast cancer risk. Int J Cancer 107(1):60–64
30. Wagner K, Hemminki K, Israelsson E, Grzybowska E, Soderberg M, Pamula J, Pekala W, Zientek H, Mielzynska D, Siwinska E et al (2005) Polymorphisms in the IGF-1 and IGFBP 3 promoter and the risk of breast cancer. Breast Cancer Res Treat 92(2):133–140
31. Mu L, Katsaros D, Wiley A, Lu L, de la Longrais IA, Smith S, Khubchandani S, Sochirca O, Arisio R, Yu H (2009) Peptide concentrations and mRNA expression of IGF-I, IGF-II and

IGFBP-3 in breast cancer and their associations with disease characteristics. Breast Cancer Res Treat 115(1):151–162
32. Deming SL, Ren Z, Wen W, Shu XO, Cai Q, Gao YT, Zheng W (2007) Genetic variation in IGF1, IGF-1R, IGFALS, and IGFBP3 in breast cancer survival among Chinese women: a report from the Shanghai Breast Cancer Study. Breast Cancer Res Treat 104(3):309–319
33. Hernandez W, Grenade C, Santos ER, Bonilla C, Ahaghotu C, Kittles RA (2007) IGF-1 and IGFBP-3 gene variants influence on serum levels and prostate cancer risk in African-Americans. Carcinog 28(10):2154–2159
34. Pechlivanis S, Wagner K, Chang-Claude J, Hoffmeister M, Brenner H, Försti A (2007) Polymorphisms in the insulin like growth factor 1 and IGF binding protein 3 genes and risk of colorectal cancer. Cancer Detect Prev 31(5):408–416
35. Feik E, Baierl A, Hieger B, Führlinger G, Pentz A, Stättner S, Weiss W, Pulgram T, Leeb G, Mach K, Micksche M, Gsur A (2010) Association of IGF1 and IGFBP3 polymorphisms with colorectal polyps and colorectal cancer risk. Cancer Causes Control 21(1):91–97
36. Terry KL, Tworoger SS, Gates MA, Cramer DW, Hankinson SE (2009) Common genetic variation in IGF1, IGFBP1 and IGFBP3 and ovarian cancer risk. Carcinog 30(12):2042–2046