Abstract

**Background:** Higher insulin-like growth factor (IGF)-1 and lower IGF binding protein (BP)-3 levels have been associated with higher common cancer risk, including breast cancer. Dietary factors, genetic polymorphisms, and the combination of both may influence circulating IGF-1 and IGFBP-3 serum concentrations.

**Methods:** From September 2011 to July 2012, we collected demographic, reproductive and dietary data on 143 women (≥40 years). We genotyped IGF-1 rs1520220 and IGFBP-3 rs2854744 and measured circulating IGF-1 and IGFBP-3 levels in serum. Covariance analyses were used to estimate the associations of serum levels of IGF-1 and IGFBP-3, and the molar ratio of IGF-1 to IGFBP-3 with rs1520220 and rs2854744 genotypes. We subsequently assessed the combined influence of genetics and diet (daily intake of protein, fat and soy isoflavones) on IGF-1 and IGFBP-3 levels.

**Results:** Among women aged less than 50 years, circulating IGF-1 serum levels were significantly lower for those with CC genotype for IGF-1 rs1520220 than levels for those with the GC or GG genotypes (in recessive model: \( P = 0.007 \)). In gene-diet analyses among these women, we found carrying CC genotype for IGF-1 rs1520220 and high soy isoflavone intake tend to be associated with lower circulating IGF-1 levels synthetically (\( P = 0.002 \)). Women with GG or GC genotypes for IGF-1 rs1520220 and with low intake of soy isoflavones had the highest levels of circulating IGF-1 (geometric mean [95% CI]: 195 [37, 1021] μg/L). Comparatively, women with both the CC genotype and high soy intake had the lowest levels of circulating IGF-1 (geometric mean [95% CI]: 120 [38, 378] μg/L).

**Conclusions:** IGF-1 serum levels are significantly lower among women with the CC genotype for IGF-1-rs1520220 to lower women’s serum IGF-1 levels more.

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Introduction

The insulin-like growth factor (IGF) -system mainly consists of IGF-1, IGF-2, IGF receptors (IGF-1R and IGF-2R), and six binding proteins (IGFBP-1-6). Within IGF system, IGF-1 and its main binding protein, IGFBP-3, are two key molecules for cellular proliferation, differentiation and apoptosis [1]. In addition to the regulation of normal cell growth, IGF systems have been implicated in carcinogenesis [2]. Results from in vivo carcinogenesis models and epidemiological studies indicate that high levels of circulating IGF-1 are associated with increased risk and progression of several common cancers, including breast [3–7], prostate [8–11], colorectal [12–15], and ovarian cancer [16–17] among others. The relationship between circulating IGFBP-3 levels and cancers have been inconsistent [14,16,18–22].

Circulating IGF-1 and IGFBP-3 levels are determined by both heritable and exogenous factors. Two twin studies estimated that approximately 40%–60% of inter-individual differences in circulating levels of IGF-1 and IGFBP-3 are attributed to heritable factors, such as single nucleotide polymorphisms (SNPs) [23–24]. Although there are many SNPs identified for IGF-1 (rs1520220, rs10735380, rs5742665, rs1549593, rs2373722, etc.) and IGFBP-3...
and 5, rs2854744, rs3110697, rs2132570, rs2270628, etc.) [25], SNP rs1520220, located in the third intron of IGF-1 gene, and rs2854744, in the promoter region of the IGFBP-3 gene, are consistently found to be associated with circulating IGF-1 and IGFBP-3 levels in Caucasians [26–29]. However, the associations of both SNPs with circulating IGF-1 and IGFBP-3 levels is not clear in the Chinese population as few studies have focused on this population. Exogenous factors, including both hormonal and nutritional, may have an important influence on circulating IGF-1 and IGFBP-3 levels [30]. Decreased levels of serum IGF-1 and IGFBP-3 were observed after chronic or acute energy restriction [31]. Several cross-sectional studies have found that serum IGF-1 concentrations are positively associated with higher total protein intake [32–34]. Soy isoflavone, a phytoestrogen, may act as selective ER modulators do by inhibiting IGF-1 concentrations or IGF system signaling [35].

In our previous studies, we mainly focused on breast cancer etiology and to be consistent with other studies in finding that nutrients, for example animal protein [36–37] may increase breast cancer risk, while high intake of soy products [38–39] may result in risk reduction. Given the associations of SNPs and dietary intake with circulating levels within the IGF system (IGF-1, IGFBP-3 and the molar ratio of IGF-1 to IGFBP-3), the hypothesis that genetic and nutritional factors may interact to effect serum concentrations of the IGF system and subsequent cancer risk warrants further investigation [30]. Several studies conducted among Caucasian women have examined the effects of nutritional factors and IGF polymorphisms on IGF serum levels [26,28,30,33–34]. However, to the best of our knowledge, no previous study has examined this issue in Chinese population in China, and previous studies did not focus on the interaction of genetic and environmental factors. In the present study, we examined the main effects of dietary intake and genetic polymorphisms on circulating IGF-1 and IGFBP-3 concentrations among women aged 40 years or older, a population with high breast cancer incidence in China (more than 42.3 per 100,000) [40]. Furthermore, we explored potential combined effects of dietary factors and gene polymorphisms on IGF-1 and IGFBP-3 concentrations.

Methods

Ethics Statement

The study protocol was approved by the Institutional Review Board at Sichuan University. All subjects provided written informed consent before completing the questionnaire survey and laboratory tests.

Study population

From September 2011 to July 2012, a total of 279 women aged 40 years and older sought the outpatient service for a physical examination from the Comprehensive Guidance Center of Women’s Health, Chengdu Women’s and Children’s Central Hospital. The women who were of Han ethnicity, had been living in Sichuan Province for over 20 years, had no history of bilateral ovariectomy, no hormonal contraceptive use, and no perimenopausal complaints. We further excluded women who declined to join the program, women with insulin-dependent diabetes mellitus, and women with diagnosed/history of malignancy, including breast, liver or ovarian cancer at baseline as these diseases may affect circulating IGF levels. Ultimately, 143 women were included in the study. To minimize possible impact of estrogens from periodical variation during the menstrual cycle on IGFs, all women were enrolled between the 3rd and 5th day from the beginning of their periods for blood sample collection.

Data collection

Information on socio-demographic and reproductive characteristics was collected using a structured questionnaire. A semi-quantitative dietary questionnaire, the Questionnaire of Health Related Dietary Habits, was designed to collect all participants’ long-term (≥5 years) dietary habits. Evaluation of reliability and structural validity for the questionnaire has been described in detail in our previous study [39]. In brief, we first calculated the total daily intake of energy, protein, fat, carbohydrate, dietary fiber, and soy isoflavones referring to the nutrient compositions listed in the Danone Institute China Diet Nutrition Evaluating System [41]. We subsequently calculated the daily intake of energy-adjusted dietary factors to prevent potential underreporting of dietary intake using residual methods [39]. According to Chinese Dietary Reference Intakes (DRI) [Chinese DRIs committee formulated in 2000] for 18–50 year old women with moderate physical labor [42], we used the following dichotimization for total daily energy (2300 kcal/day), protein (70 g/day) and fat intake (77 g/day). For those without recommended levels of dietary intake, the means of dietary intakes (167.0 g/day for carbohydrate, 19.8 g/day for dietary fiber and 11.6 mg/day for soy isoflavones) were selected as cut-off values of high/low intake.

Genotype analyses

Whole blood samples (5 mL) were obtained from participants via venipuncture into tubes containing ethylenediaminetetraacetic acid. Samples were stored at −20°C until DNA extraction. Genomic DNA was extracted from whole blood using TIANamp Blood DNA Kit (TIANGEN, Beijing). IGF-1 rs1520220 and IGFBP-3 rs2854744 were genotyped with TaqMan assays which were purchased from ABI (Applied Biosystems, Foster City, CA). All TaqMan assays were performed with the ABI 7500 thermal cycler (Applied Biosystems, Foster City, CA). According to manufacturers’ instructions. Duplicate aliquots from each blood sample were genotyped analyses. Coefficients of variation (CVs) for duplication were less than 10%. The intra- and inter-assay coefficients of variation were 4.9% and 12.8%, respectively, for IGF-1 at a concentration of 110 ng/ml. Intra and inter-assay coefficients of variation were 4.9% and 5.4%, respectively, for IGFBP-3 at a concentration of 4,900 ng/ml. The molar ratio, which may estimate the biologically active fraction of IGF-1, was calculated based on equation 1:

$$\text{IGF–1 : IGFBP–3 molar ratio} = \frac{(C_1 \times 0.13)}{(C_2 \times 0.036) \times 1000}$$
C1 and C2 are concentrations of IGF-1 and IGFBP-3, respectively. For IGF-1, 1 ng/ml is equal to 0.130 nM, and for IGFBP-3, 1 ng/ml is equal to 0.036 nM.

To ensure blinded laboratory analyses, participants were assigned a unique random ID number at each clinic visit.

Statistical analyses

We checked the Hardy–Weinberg equilibrium (HWE) among all women via Chi-square test. The measured total IGF-1 and IGFBP-3 levels were log transformed to reduce departures from the normal distribution and then described using the geometric mean (95% confidence interval [CI]). After making a scatter plot of IGF component levels (i.e. IGF-1, IGFBP-3, and the molar ratio of IGF-1 to IGFBP-3) by age, we found that IGF levels were relatively more centralized among women aged less than 50 years. Considering differences in sex steroid levels between two age groups, which may affect circulating IGF levels, we divided the participants into two subgroups for sub-group analyses: <50 years and ≥50 years. Independent-sample T-tests and Chi-square tests/Fisher’s exact tests were used to compare the demographic characteristics, and reproductive and dietary factors between age subgroups. Independent-sample T-tests were used to compare geometric means of IGF-1, IGFBP-3, and the molar ratio of IGF-1 to IGFBP-3 (equation 1), for each exposure of interest. Factors that were significantly associated with IGFBP-3 component levels were then adjusted for as potential confounders in the covariance analyses to test the differences of IGF component levels within gene-diet exposure groups. Referring to results from previous studies that noted carrying the CC genotype for IGF-1 rs1520220 was associated with lower IGF-1 levels in breast tissue among Chinese women [43], and that circulating levels of IGFBP-3 were significantly lower for CC or CA genotype carriers for IGF-1 rs2854744 than AA allele carriers [44], we analyzed the effects of circulating IGFBP-3 levels in women with low soy intake (groups 1 and 2: (geometric mean [95% CI]: 195 [37, 1021] μg/L), but these differences

IGF-1 rs1520220/IGFBP-3 rs2854744 genotypes and circulating IGF component levels

Among all women, the frequency of the C allele for IGF-1 rs1520220 and the C allele for IGFBP-3 rs2854744 were 56.6% and 27.1%, respectively. Genotypes of IGF-1 rs1520220 and IGFBP-3 rs2854744 did not deviate from HWE (IGF-1 rs1520220: χ² = 3.04, P = 0.08; IGFBP-3 rs2854744: χ² = 2.29, P = 0.13). Table 3 presents covariance analyses results of IGF-1 rs1520220 and IGFBP-3 rs2854744 genotypes in relation to circulating levels of IGF components. Among women <50 years, circulating IGF-1 levels were significantly lower for those carrying the CC genotype for IGF-1 rs1520220 than IGF-1 levels for those carrying GC or GG genotypes (recessive model: P = 0.007). We did not observe any association between IGFBP-3 rs2854744 genotypes and circulating IGFBP-3 level or the molar ratio.

Combination of IGF-1 rs1520220/IGFBP-3 rs2854744 genotypes and daily intake of energy-adjusted soy isoflavones and protein on circulating IGF component levels among women <50 years old

For women <50 years, those with both low intake of soy isoflavones and at least one variant G allele for IGF-1 rs1520220 had the highest levels of circulating IGF-1 (geometric mean [95% CI]: 195 [37, 1021] μg/L). Women who either had high intake of dietary soy or were homozygous for the major C allele had significantly lower levels of circulating IGF-1 (geometric mean [95% CI]: 160 [34, 753] μg/L and 167 [38, 723] μg/L, respectively). Women who had both high soy intake and were homozygous for the major C allele had the lowest levels of circulating IGF-1 (geometric mean [95% CI]: 120 [38, 378] μg/L) (Table 4, P = 0.002). As for combination of IGFBP-3 rs2854744 genotypes and soy isoflavone intake, we found higher IGFBP-3 levels in women with low soy intake (groups 1 and 2: geometric mean [95% CI]: 1832 [503, 6671] μg/L and 2014 [613, 6613] μg/L, respectively) than those women with high soy intake (groups 3 and 4: geometric mean [95% CI]: 1654 [663, 4124] μg/L and 1735 [641, 4096] μg/L, respectively), but these differences appear to be driven primarily by soy intake, as the overall effect of diet and genetics was not significant (P = 0.40). Although protein intake were found to be related to IGF system variables (Table 2), combinations of IGF-1 rs1520220/IGFBP-3 rs2854744 genotypes and daily intake of energy-adjusted protein were not associated with IGFBP-3 component levels (P = 0.05).

While dietary fiber intake seems to be positively related to the molar ratio of IGF-1 to IGFBP-3 in the ≥50 age group (Table 2), we did not analyze combinations of IGF-1 rs1520220/IGFBP-3...
rs2854744 genotypes and dietary fiber intake due to limited sample size in this age group.

**Discussion**

In this study, we aimed to reveal the genetic and dietary determinants of IGF-1 and IGFBP-3 in our population of Chinese women aged 40 years or older. We found that carrying CC genotype for IGF-1 rs1520220 may work alone or interact with dietary intake, for example, soy isoflavone intake, to decrease serum IGF-1 levels among women aged 50 years old. Since relatively high circulating IGF-1 expression, synthesis, and secretion, resulting in altered serum IGF levels; ii. Certain dietary factors (i.e. protein and fat intake) could affect IGFBP levels, and may also influence the binding ability of IGF-1 to IGFBP-1 by competing for active sites; iii. Dietary factors may indirectly affect the IGF system through interaction with genetic factors.

Soy food has consistently been characterized to have an anticancer effect [50], particularly in Asian populations where soy intake is relatively high. In vitro studies have shown that pharmacological doses of genistein, the main composition of soy isoflavones, may stimulate IGF-1R signaling human breast cancer cells [51]. Although we did not find associations between soy intake and serum IGF-1 as previous studies did [52], we observed that high soy intake enhanced the association between carrying CC genotype for IGF-1 rs1520220 and lower circulating IGF-1 levels among women aged <50 years. Since relatively high circulating IGF-1 concentration is associated with increased cancer risk [18-19], modulation of IGF-1 levels by soy isoflavone intake may be implemented as a risk reduction mechanism, particularly for breast cancer as reported in our previous studies [38-39]. We also hypothesized that IGFBP-3 polymorphisms may potentially interact with dietary intake, for example, soy isoflavone intake,

**Table 1.** Descriptive statistics of demographic, reproductive and insulin-like growth factors by age status.

| Variables                                      | <50 years | ≥50 years | P value<sup>a</sup> |
|------------------------------------------------|-----------|-----------|---------------------|
| BMI (kg/m<sup>2</sup>)<sup>b</sup>            | 103       | 38        | 0.72                |
| WHR<sup>b</sup>                                | 89        | 35        | 0.21                |
| Age at menarche (years)<sup>b</sup>            | 103       | 37        | 0.08                |
| Passive smoking, yes, n (%)                    | 69        | 25        | 0.73                |
| Parity, ≥2, n (%)                              | 16        | 7         | 0.69                |
| Breast feeding, ≥3 months, n (%)               | 75        | 22        | 0.66                |
| Total energy intake (kcal/day)<sup>b</sup>     | 104       | 39        | 0.68                |
| Protein intake (g/day)<sup>a, b</sup>          | 104       | 39        | 0.17                |
| Fat intake (g/day)<sup>a, b</sup>              | 104       | 39        | 0.33                |
| Carbohydrate intake (g/day)<sup>a, b</sup>     | 104       | 39        | 0.47                |
| Dietary fiber intake (g/day)<sup>a, b</sup>    | 104       | 39        | 0.30                |
| Soy isoflavone intake (mg/day)<sup>a, b</sup>  | 104       | 39        | 0.32                |
| Insulin-like growth factor-1 (µg/L)<sup>c</sup>| 104       | 39        | 0.25                |
| Insulin-like growth factor binding protein-3 (µg/L)<sup>c</sup> | 104 | 39 | 0.38 |
| IGF-1:IGFBP-3 molar ratio<sup>c</sup>          | 104       | 39        | 0.09                |

<sup>a</sup> The dietary key nutrient intakes, including protein, fat, carbohydrates, dietary fiber, and soy isoflavones are adjusted for energy by residual method; <sup>b</sup> mean ± standard deviation; <sup>c</sup> Geometric mean (95% confidence interval); <sup>d</sup> P value is based on T test for continuous variables and χ<sup>2</sup> test or Fisher’s exact test for categorical variables. Waist to hip ratio, WHR; Body mass index, BMI.

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Table 2. Relationship between geometric mean (95% CI) of insulin-like growth factor component levels with dietary intake.

| Variables                        | N   | IGF-1 (µg/L) | IGFBP-3 (µg/L) | IGF-1:IGFBP-3 |
|---------------------------------|-----|--------------|---------------|---------------|
| **Overall**                     |     |              |               |               |
| Total energy intake (kcal/day)  | <2300| 130          | 181 (36,906)  | 1782 (580,5476) | 367 (67,2015) |
|                                 | ≥2300| 13           | 134 (36,498)  | 1874 (469,7492) | 258 (66,1009) |
| P value                         |     | 0.20         | 0.77          | 0.16          |
| Protein intake (g/day)          | <70  | 93           | 138 (35,913)  | 1694 (525,5467) | 380 (67,2161) |
|                                 | ≥70  | 50           | 173 (38,790)  | 1984 (679,5796) | 315 (66,1499) |
| P value                         |     | 0.85         | 0.12          | 0.22          |
| Fat intake (g/day)              | <77  | 100          | 182 (34,981)  | 1834 (585,5746) | 359 (65,1998) |
|                                 | ≥77  | 43           | 163 (42,633)  | 1692 (534,5362) | 348 (69,1757) |
| P value                         |     | 0.45         | 0.45          | 0.85          |
| Carbohydrate intake (g/day)     | <167.0| 70          | 163 (35,756)  | 1747 (577,5286) | 337 (64,1779) |
|                                 | ≥167.0| 73         | 190 (37,978)  | 1833 (561,5986) | 375 (68,2060) |
| P value                         |     | 0.25         | 0.62          | 0.46          |
| Dietary fiber intake (g/day)    | <19.8| 67           | 155 (37,647)  | 1793 (548,5873) | 312 (61,1608) |
|                                 | ≥19.8| 76           | 197 (36,1078) | 1787 (587,5439) | 399 (73,2180) |
| P value                         |     | 0.07         | 0.97          | 0.09          |
| Soy isoflavone intake (mg/day)  | <11.6| 90           | 139 (36,1022) | 1870 (541,6465) | 372 (63,2193) |
|                                 | ≥11.6| 53           | 151 (37,622)  | 1662 (642,4303) | 329 (72,1504) |
| P value                         |     | 0.09         | 0.21          | 0.39          |
| **<50 years**                   |     |              |               |               |
| Total energy intake (kcal/day)  | <2300| 92           | 173 (37,816)  | 1840 (617,5485) | 340 (64,1814) |
|                                 | ≥2300| 12           | 133 (34,526)  | 1821 (435,7620) | 264 (64,1086) |
| P value                         |     | 0.28         | 0.95          | 0.33          |
| Protein intake (g/day)          | <70  | 68           | 163 (38,711)  | 1700 (542,5334) | 346 (63,1917) |
|                                 | ≥70  | 36           | 178 (34,934)  | 2129 (742,6111) | 302 (65,1392) |
| P value                         |     | 0.59         | 0.06          | 0.43          |
| Fat intake (g/day)              | <77  | 68           | 176 (34,905)  | 1924 (623,5944) | 330 (59,1844) |
|                                 | ≥77  | 36           | 154 (41,577)  | 1684 (547,5188) | 330 (72,1521) |
| P value                         |     | 0.41         | 0.26          | 0.99          |
| Carbohydrate intake (g/day)     | <167.0| 55         | 158 (34,724)  | 1774 (581,5421) | 321 (67,1552) |
|                                 | ≥167.0| 49         | 180 (38,849)  | 1911 (607,6022) | 341 (60,1492) |
| P value                         |     | 0.39         | 0.31          | 0.72          |
| Dietary fiber intake (g/day)    | <19.8| 54           | 153 (36,667)  | 1734 (508,5917) | 319 (65,1575) |
|                                 | ≥19.8| 50           | 186 (38,911)  | 1957 (714,5363) | 342 (62,1905) |
| P value                         |     | 0.22         | 0.29          | 0.68          |
| Soy isoflavone intake (mg/day)  | <11.6| 63           | 184 (38,889)  | 1934 (567,6600) | 344 (64,1847) |
|                                 | ≥11.6| 41           | 146 (34,620)  | 1699 (657,4393) | 311 (62,1551) |
| P value                         |     | 0.14         | 0.26          | 0.55          |
| **≥50 years**                   |     |              |               |               |
| Total energy intake (kcal/day)  | <2300| 38           | 202 (35,1158) | 1649 (499,5445) | 443 (78,2512) |
|                                 | ≥2300| 1            | 140           | 2650          | 191          |
| P value                         |     | –            | –             | –             |
| Protein intake (g/day)          | <70  | 25           | 226 (32,1618) | 1677 (471,5971) | 486 (84,2821) |
|                                 | ≥70  | 14           | 162 (33,496)  | 1655 (567,4835) | 353 (66,1879) |
| P value                         |     | 0.19         | 0.95          | 0.28          |
| Fat intake (g/day)              | <77  | 32           | 196 (33,1180) | 1656 (517,5305) | 428 (79,2310) |
|                                 | ≥77  | 7            | 220 (50,968)  | 1729 (431,6945) | 459 (58,3662) |
| P value                         |     | 0.76         | 0.87          | 0.85          |
| Carbohydrate intake (g/day)     | <167.0| 15         | 183 (36,917)  | 1648 (548,4962) | 400 (55,2905) |
|                                 | ≥167.0| 24         | 212 (35,1306) | 1682 (476,5943) | 456 (93,2244) |
and then influence circulating IGF component levels. However, we found negative results, which may be due partly to the limited sample in our study. This hypothesis deserves further study with a larger sample size.

Total energy and dietary protein have been shown to be positively associated with serum IGF levels [32–34]. We observed no associations between total energy and IGF component levels, although there was a borderline significant positive association for dietary protein and IGFBP-3, it was limited to women aged 50 years (Table 2). In this age group, positive results disappeared when we combined protein intake and IGF-1 rs1520220/IGFBP-3 rs2854744 genotypes. Few studies paid attention to the relationship of dietary fiber intake and circulating IGF levels. Gannet al. 2005 found low fat, high fiber dietary intake didn’t change circulatory IGF-1 or IGFBP-3 levels compared to the usual diet among women in Chicago after a intervention period of 12 menstrual cycles [53]. However, the main effect of dietary fiber intake isn’t clear yet. In our study, while we observed a possible relationship of high dietary fiber intake with increased molar ratio of IGF-1 to IGFBP-3 in the \( \geq 50 \) age group, relatively limited sample (\( N = 39 \)) may have limited our ability to ascertain the association of dietary fiber intake combined with IGF-1 rs1520220/IGFBP-3 rs2854744 genotypes. We believe that the effects of fiber intake on IGF system relationship of dietary fiber intake and circulating IGF levels.

### Table 2. Cont.

| Variables                   | N   | IGF-1 (\( \mu \)g/L) | IGFBP-3 (\( \mu \)g/L) | IGF-1:IGFBP-3 |
|-----------------------------|-----|----------------------|------------------------|--------------|
| \( <19.8 \) g/day           |     | 0.61                 | 0.92                   | 0.66         |
| \( \geq 19.8 \) g/day       |     | 0.30                 | 0.12                   | 0.03         |
| Soy isoflavone intake (mg/day) |     | 0.46                 | 0.59                   | 0.66         |

| Variables                   | N   | IGF-1 (\( \mu \)g/L) | IGFBP-3 (\( \mu \)g/L) | IGF-1:IGFBP-3 |
|-----------------------------|-----|----------------------|------------------------|--------------|
| IGF-1 GG+ CG                | 92  | 191 (35,1039)        | 1819(574,5765)          | 380(68,2115)  |
| CC                          | 51  | 152(39,589)          | 1738(558,5412)          | 316(63,1576)  |
| P value                     |     | 0.10                 | 0.66                   | 0.22         |
| IGFBP-3 CC+CA               | 63  | 161(35,731)          | 1723(566,5251)          | 337(57,2009)  |
| AA                          | 79  | 187(36,963)          | 1838(567,5957)          | 368(74,1831)  |
| P value                     |     | 0.23                 | 0.70                   | 0.57         |

### Table 3. Relationship between geometric mean (95% CI) of insulin-like growth factor component levels with IGF-1 and IGFBP-3 genotypes.

| Variables                   | N   | IGF-1 (\( \mu \)g/L) | IGFBP-3 (\( \mu \)g/L) | IGF-1:IGFBP-3 |
|-----------------------------|-----|----------------------|------------------------|--------------|
| Overall                     |     |                      |                        |              |
| IGF-1 GG+ CG                | 66  | 180(36,8980)         | 1907(634,5731)         | 341(61,1900)  |
| CC                          | 38  | 149(37,597)          | 1724(530,5605)         | 313(67,1450)  |
| P value                     |     | 0.007                | 0.16                   | 0.46         |
| IGFBP-3 CC+CA               | 45  | 164(34,800)          | 1759(558,5546)         | 337(55,2075)  |
| AA                          | 59  | 171(38,773)          | 1900(621,5812)         | 325(71,1489)  |
| P value                     |     | 0.76                 | 0.44                   | 0.80         |

### <50 years

| Variables                   | N   | IGF-1 (\( \mu \)g/L) | IGFBP-3 (\( \mu \)g/L) | IGF-1:IGFBP-3 |
|-----------------------------|-----|----------------------|------------------------|--------------|
| IGF-1 GG+ CG                | 26  | 224(34,1478)         | 1616(454,5754)         | 501(99,2534)  |
| CC                          | 13  | 160(44,591)          | 1781(629,5044)         | 325(50,2116)  |
| P value                     |     | 0.29                 | 0.97                   | 0.25         |

### \( \geq 50 \) years

| Variables                   | N   | IGF-1 (\( \mu \)g/L) | IGFBP-3 (\( \mu \)g/L) | IGF-1:IGFBP-3 |
|-----------------------------|-----|----------------------|------------------------|--------------|
| IGF-1 GG+ CG                | 18  | 153(39,597)          | 1638(575,4670)         | 337(59,1945)  |
| CC                          | 20  | 244(36,1667)         | 1666(434,6396)         | 528(99,2823)  |
| P value                     |     | 0.14                 | 0.85                   | 0.25         |

Insulin-like Growth Factor, IGF; Insulin-like Growth Factor Binding Protein, IGFBP.

\( ^a \): Models among women \( <50 \) years were adjusted for WHR (waist hip ratio), passive smoking and protein intake; \( ^b \): Models among women \( \geq 50 \) years were adjusted for age at menarche and dietary fiber intake.

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### Table 4. Relationship between geometric mean (95% CI) of insulin-like growth factor component levels with IGF-1 and IGFBP-3 genotypes combined soy isoflavone and protein intake among women <50 years old.

| Group | Dietary intake | IGF-1 genotypes | N   | IGF-1 (μg/L) | IGFBP-3 (μg/L) | IGF-1: IGFBP-3 | P value a |
|-------|----------------|-----------------|-----|--------------|----------------|----------------|------------|
| **Soy isoflavone intake** | | | | | | | |
| 1 low (<11.6) | GG+ CG | 38 | 195 (37,1021) | 206 (61,76916) | 342 (60,1932) | CC+CA | 27 | 174 (39,776) | 183 (50,361) | 344 (64,1832) | 0.002 |
| 2 low (<11.6) | CC | 25 | 167 (38,723) | 174 (67,1779) | 345 (67,1779) | AA | 36 | 191 (36,997) | 201 (61,6613) | 343 (61,1901) | |
| 3 high (≥11.6) | GG+ CG | 28 | 160 (34,753) | 170 (68,1917) | 338 (60,1917) | CC+CA | 18 | 149 (26,850) | 165 (63,4124) | 326 (61,2576) | |
| 4 high (≥11.6) | CC | 13 | 120 (38,378) | 167 (500,4862) | 257 (71,931) | AA | 23 | 144 (31,480) | 173 (61,4696) | 299 (92,972) | |
| **Protein intake** | | | | | | | |
| 1 low | GG+ CG | 44 | 176 (38,830) | 178 (5780,5477) | 358 (61,2109) | CC+CA | 31 | 159 (32,802) | 159 (485,5240) | 361 (53,2461) | |
| 2 low | CC | 24 | 141 (38,522) | 156 (478,5107) | 326 (65,1649) | AA | 37 | 166 (42,650) | 179 (591,5436) | 335 (72,1560) | |
| 3 high (≥70) | GG+ CG | 22 | 187 (32,1083) | 218 (786,6091) | 309 (61,1569) | CC+CA | 14 | 175 (37,83) | 218 (836,5696) | 289 (59,1425) | |
| 4 high (≥70) | CC | 14 | 164 (35,769) | 204 (657,6346) | 290 (70,1203) | AA | 22 | 180 (31,1037) | 209 (676,6499) | 310 (68,1419) | |

Insulin-like Growth Factor, IGF; Insulin-like Growth Factor Binding Protein, IGFBP.

a: Models among women <50 years were adjusted for WHR (waist hip ratio), passive smoking and protein intake.

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levels and corresponding cancer risk deserve further study. To our knowledge, our study is the first to explore the combined-influence of IGF-1 and IGFBP-3 genotypes on IGF-1 levels and breast cancer risk: findings from the Breast and Prostate Cancer Cohort Consortium [EPC3]. PlLoS One 37: 2527.

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