**Null Results in Brief**

**IGF-I and IGF-II Genetic Variation and Breast Cancer Risk in Chinese Women: Results from the Shanghai Breast Cancer Study**

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**Introduction**

Insulin-like growth factor (IGF)-I and IGF-II have been implicated in breast tumorigenesis due to their ability to stimulate mitogenesis and promote differentiation and their key role in mammary gland cell proliferation and survival (1-3). It has been reported that genetic variations in the gene encoding IGF-I are associated with levels of the protein and, as a consequence, may alter breast cancer risk (4, 5). Results of recent studies investigating the role of IGF-I and IGF-II genetic polymorphisms in breast cancer risk have been inconsistent (4-10). The majority of the previous studies, including one from our own group, have focused on the (CA)n repeat in the promoter of the IGF-I gene (7-9, 11), whereas fewer have characterized common variants across the IGF-I and IGF-II genes in relationship to breast cancer susceptibility (4-6). To further assess the role of genetic variation in these genes, we evaluated the association between 23 single-nucleotide polymorphisms (SNP) in the IGF-I and IGF-II genes and breast cancer risk among participants of the Shanghai Breast Cancer Study, a population-based case-control study of incident breast cancer in urban Shanghai.

**Materials and Methods**

**Study Population.** Detailed study methods have previously been published (12). Briefly, this study is a population-based case-control study of incident breast cancer in Chinese women in urban Shanghai, ages 25 to 64 years, who were recruited from 1996 to 1998. Of 1,602 eligible cases identified by the Shanghai Cancer Registry and 1,724 age-frequency matched controls identified using the Shanghai Resident Registry, 1,459 (91.1%) cases and 1,556 (90.3%) controls participated in the study. Approximately, 82% (1,193) of cases and 84% (1,310) of controls provided blood samples. Genomic DNA was extracted from buffy coats using the Puregene DNA Purification Kit (Gentra Systems) following the manufacturer’s protocol. There were no differences in the distribution of demographic and risk factors between individuals who did and did not have DNA available for genotyping (13).

**SNP Selection and Genotyping.** To comprehensively evaluate the association between the IGF-I and IGF-II gene polymorphisms and breast cancer risk, we included haplotype tagging SNPs and potentially functional variants. Potentially functional and nonsynonymous SNPs were identified from literature reports and physical location (promoter or intron/exon boundary region) using the database SNPper3 or dbSNP.4 Haplotype tagging SNPs were identified from the Han Chinese data in the HapMap project for each gene plus flanking 5-kb region with the pairwise $r^2 \geq 0.9$ and minor allele frequency $\geq 0.05$. The above-mentioned potentially functional SNPs were forced into the haplotype tagging SNP list. A total of 20 IGF-I and 3 IGF-II SNPs were included in the present study. The SNPs were genotyped by running the 5’ nuclease TaqMan allelic discrimination assay (Applied Biosystems) and with the Affymetrix MegAllele Targeted Genotyping System (Affymetrix). The concordance rates for the quality control samples were 97% and 99% for TaqMan and Affymetrix methods, respectively.

**Statistical Analysis.** The $\chi^2$ test was used to compare the distributions of IGF-I and IGF-II alleles and genotypes in the cases and controls. The exact $\chi^2$ goodness-of-fit test was used to evaluate whether genotype distributions were in Hardy-Weinberg equilibrium. Odds
ratios and 95% confidence intervals were estimated using logistic regression. All analyses were adjusted for age, with additional adjustment for other confounding factors including menopausal status, age at menarche, and age at first full-term pregnancy. Haplotypes were generated using the Haplov program (14), which uses an expectation-maximization algorithm to estimate haplotypes. Odds ratios and 95% confidence intervals for the association between haplotypes and breast cancer risk were generated using the Hapstat program (15). Associations between genotypes, haplotypes, and breast cancer risk were evaluated under additive, dominant, and recessive genetic models.

**Results**

The distributions of selected demographic characteristics and major risk factors for breast cancer among the cases and controls have been presented elsewhere (13). Briefly, the mean age was 47.7 ± 8.0 years among cases and 47.2 ± 8.7 years among controls. As compared with controls, cases were significantly more likely to have a history of fibroadenoma (9.8% versus 5.1%), a younger age at menarche (14.5 versus 14.7 years), an older age at menopause (48.2 versus 47.5 years), and a higher body mass index.

Table 1 details the polymorphisms in the *IGF-I* and *IGF-II* genes and their association with breast cancer risk. Genotype frequencies were comparable to those for the Chinese Han population included in HapMap. With the exception of one SNP (rs2288377), all genotype frequencies were found to be consistent with Hardy-Weinberg equilibrium among controls. None of the 23 polymorphisms investigated were significantly associated with breast cancer risk when evaluated under additive, dominant, and recessive models. Haplotype blocks were estimated for both *IGF-I* and *IGF-II* genes, and no association between any of the haplotypes and altered breast cancer risk was observed. Table 2 presents results under the additive model. Findings were similar under dominant and recessive models (data not shown). Potential modifying effects by traditional risk factors were investigated on the relationship of the single polymorphisms and haplotypes with breast cancer risk. No evidence was found for an interaction between any of the genetic variants or haplotypes with age, menopausal status, body mass index, or waist-to-hip ratio (data not shown).

**Discussion**

The results from this study suggest that common genetic variants in the *IGF-I* and *IGF-II* genes do not play a significant role in breast cancer risk among Chinese women. One of the main strengths of this study is its comprehensive and systematic approach to characterizing variation in *IGF-I* and *IGF-II*. We selected SNPs with known or potential function as well tagging SNPs to provide sufficient coverage across the gene. In addition,
The the large sample size provided sufficient power (≥80%) to detect a minimum odds ratio of ≥1.25 (assuming minor allele frequency ≥10%; x = 0.05 on the log-additive scale) and allowed evaluation of moderate or higher interactions between genetic polymorphisms and traditional breast cancer risk factors (16).

Although a number of studies have investigated the association between the (CA)n repeat polymorphism in the *IGF-I* promoter and breast cancer risk with inconsistent results (7-9, 11), only three evaluated the role of multiple common genetic variants across the *IGF-I* gene in breast cancer incidence (4-6). Our results are consistent with those observed among four other ethnic groups in a European Prospective Investigation into Cancer and Nutrition study conducted primarily in the Caucasian population found a borderline significant association with breast cancer risk for the rs2162679 polymorphism in the *IGF-I* gene (odds ratio, 0.57; 95% confidence interval, 0.34-0.97 for the homozygous variant genotype) but not the four other SNPs investigated (rs35765, rs35767, rs6220, and rs6214). With respect to *IGF-II*, ours is the first study to evaluate polymorphisms across the gene in relation to breast cancer susceptibility.

Our results indicate that common genetic variants in the *IGF-I* or *IGF-II* genes may not appreciably alter breast cancer risk among Chinese women. However, we cannot rule out the possibility that some genetic variants may exert their effect through interactions with genetic polymorphisms in other genes or certain lifestyle factors. These interactions can be addressed in future studies with large sample size.

### Acknowledgments

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### References


### Table 2. Association between *IGF-I* and *IGF-II* haplotypes and breast cancer risk

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Frequency</th>
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<td>TCCA</td>
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<tr>
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<td>16.7</td>
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<tr>
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<tr>
<td>CTC</td>
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<td>15.8</td>
</tr>
</tbody>
</table>

Abbreviations: OR, odds ratio; 95% CI, 95% confidence interval.
*Additive model adjusted for age, menopausal status, age at menarche, and age at first full-term pregnancy.

1. rs10860861, rs6219, rs2946834, and rs5742726.
2. rs6218 and rs6220.
3. rs4764697 and rs2195239.
4. rs2288377 and rs35767.
5. rs1520220, rs10860861, rs6219, rs2946834, and rs5742726.
6. rs2195239, rs6218 and rs6220.
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