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IGF-1 and *IGF-2* 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 1 (IGF-1) and 2 (IGF-2), have been implicated in breast tumorigenesis due to their ability to stimulate mitogenesis, 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-1 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 *IGF1* and *IGF2* 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 *IGF1* gene[7–9,11], while fewer have characterized common variants across the *IGF1* and *IGF2* 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 (SNPs) in the *IGF1* and *IGF2* 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 been published previously[12]. Briefly, this study is a population-based case-control study of incident breast cancer in Chinese women aged 25–64 in urban Shanghai who were recruited from 1996–1998. 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 cases (91.1%) and 1,556 controls (90.3%) participated in the study. Approximately, 82% of cases (1,193) and 84% of controls (1,310) provided blood sample. Genomic DNA was extracted from buffy coats using the Puregene® DNA Purification Kit (Gentra Systems, Minneapolis, MN) following the manufacturers 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].

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SNP Selection and Genotyping

In order to comprehensively evaluate the association between the *IGF1* and *IGF2* 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 SNPper (http://snpper.chip.org/bio/snpper-enter), or dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/). Haplotype tagging SNPs (htSNP) were identified from the Han Chinese data in the HapMap project for each gene plus flanking 5 kb region with the pair-wise $r^2 \geq 0.9$ and MAF ≥ 0.05. The above mentioned potentially functional SNPs were forced into the htSNP list. A total of 20 *IGF1* and three *IGF2* SNPs were included in the present study. The SNPs were genotyped by running the 5'nuclease TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA) and with the Affymetrix MegAllele Targeted Genotyping System (Affymetrix, Santa Clara, CA). The concordance rate for the quality control samples were 97% and 99% for Taqman and Affymetrix methods respectively.

Statistical Analysis

The χ^2 test was used to compare the distributions of *IGF1* and *IGF2* alleles and genotypes in the cases and controls. The exact χ^2 goodness-of-fit test was used to evaluate whether genotype distribution 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 Haploview program[14] which employs an expectation-maximization algorithm to estimate haplotypes. Odds ratios and 95% CIs for the association between haplotypes and breast cancer risk were generated using the Haplostat program[15]. Associations between genotypes, haplotypes, and breast cancer risk were evaluated under additive, dominant, and recessive genetic modes.

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 to controls, cases were significantly more likely to have a history of fibroadenoma (9.8% vs 5.1%), a younger age at menarche (14.5 yrs. vs. 14.7 yrs.), an older age at menopause (48.2 yrs. vs. 47.5 yrs.) and a higher BMI.

Table 1 details the polymorphisms in the *IGF1* and *IGF2* 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 we investigated were significantly associated with breast cancer risk when evaluated under additive, dominant, and recessive models. Haplotype blocks were estimated for both *IGF1* and *IGF2* 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, BMI, or waist-hip ratio (data not shown).

Discussion

The results from this study suggest that common genetic variants in the *IGF1* and *IGF2* genes do not play a significant role in the breast cancer risk among Chinese women. One of the main strengths of this study is its comprehensive and systematic approach to characterizing variation in *IGF1* and *IGF2*. We selected SNPs with known or potential function as well tagging SNPs to provide sufficient coverage across the gene. In addition, the large sample size provided sufficient power (≥80%) to detect a minimum OR of ≥ 1.25 (assuming minor allele frequency 10%, α=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 polymorphisms in the *IGF1* promoter and breast cancer risk with inconsistent results[7–9,11], only three evaluated the role of multiple common genetic variants across the *IGF1* gene in breast cancer incidence[4–6]. Our results are consistent with those observed among four other ethnic groups in a multiethnic cohort which found no significant association between *IGF1* variants or haplotypes and breast cancer risk[5]. In an investigation of nine *IGF1* polymorphisms, Al-Zahrani et.al. found that the variant allele in *rs1520220* (a SNP not evaluated in our study, but in high LD with *rs6220*), although significantly related with reduced plasma IGF-1 levels, was associated with an increased risk of breast cancer[4]. This finding is unexpected given the tumor-promoting effect of IGF-1. Results from the European Prospective Investigation into Cancer and Nutrition (EPIC) study conducted primarily in the Caucasian population found a borderline significant association with breast cancer risk for the *rs2162679* polymorphism in the *IGF1* gene (OR=0.57, 95% CI=0.34–0.97 for the homozygous variant genotype), but not the four other SNPs investigated (*rs35765, rs35767, rs6220, rs6214)*. With respect to *IGF2*, 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 *IGF1* or *IGF2* 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 the other genes or certain lifestyle factors. These interactions can be addressed in future studies with large sample size.

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Table 1

Association between genetic polymorphisms in the *IGF-1* and *IGF-2* genes and breast cancer risk

Marker	Allele*	Location	MAF†	Genotype frequency ‡			p-value§	Odd ratios (95% CIs)‖		
				AA	AB	BB		AA	AB	BB
IGF-1										
rs9919733	**A**/G	Promoter	0.28	0.52	0.41	0.07	0.69	1.0	0.9 (0.7–1.0)	1.1 (0.8–1.5)
rs35767	**C**/T	Promoter	0.34	0.42	0.47	0.11	0.20	1.0	0.8 (0.7–1.0)	1.0 (0.8–1.4)
rs12579108	**A**/T	Promoter	0.28	0.49	0.43	0.07	0.50	1.0	0.8 (0.7–1.0)	1.2 (0.8–1.6)
rs2288377	**C**/A	Promoter	0.29	0.50	0.42	0.08	0.04	1.0	0.8 (0.7–1.0)	1.1 (0.8–1.5)
rs2162679	**A**/G	intron	0.35	0.41	0.48	0.11	0.19	1.0	0.8 (0.7–1.0)	1.0 (0.8–1.4)
rs5742615	**C**/A	intron	0.27	0.52	0.41	0.07	0.24	1.0	1.1 (0.8–1.5)	1.0 (0.8–1.4)
rs12821878	**G**/A	intron	0.05	0.91	0.08	0.01	0.23	1.0	1.0 (0.8–1.2)	1.3 (0.4–4.7)
rs7956547	**T**/C	intron	0.16	0.71	0.26	0.03	0.68	1.0	0.8 (0.7–1.0)	1.2 (0.7–2.0)
rs2195239	**G**/C	intron	0.43	0.32	0.50	0.18	0.57	1.0	1.0 (0.8–1.2)	0.9 (0.7–1.2)
rs4764697	**C**/T	intron	0.16	0.71	0.26	0.03	0.94	1.0	0.9 (0.7–1.1)	1.0 (0.6–1.7)
rs5742692	**T**/C	intron	0.26	0.53	0.41	0.06	0.18	1.0	0.9 (0.8–1.1)	1.1 (0.8–1.6)
rs978458	**C**/T	intron	0.42	0.33	0.49	0.18	0.81	1.0	0.8 (0.7–1.0)	1.0 (0.8–1.3)
rs6220	**T**/C	3’ UTR	0.42	0.33	0.49	0.18	0.94	1.0	1.0 (0.8–1.2)	1.0 (0.8–1.3)
Rs6218	**T**/C	3’ UTR	0.25	0.55	0.39	0.06	0.59	1.0	1.0 (0.8–1.1)	1.1 (0.8–1.7)
rs6214	**G**/A	3’ UTR	0.48	0.27	0.50	0.23	0.88	1.0	1.0 (0.8–1.2)	0.9 (0.7–1.1)
rs5742723	**C**/A	3’ UTR	0.28	0.51	0.42	0.07	0.10	1.0	0.9 (0.8–1.1)	1.2 (0.8–1.6)
rs2946834	**C**/T	3’ UTR	0.46	0.29	0.50	0.21	0.54	1.0	1.0 (0.8–1.2)	1.0 (0.8–1.3)
rs6219	**C**/T	3’ UTR	0.16	0.70	0.27	0.03	0.49	1.0	1.0 (0.8–1.2)	0.9 (0.6–1.5)
rs10860861	**T**/C	3’ UTR	0.38	0.38	0.47	0.15	0.93	1.0	1.0 (0.8–1.2)	1.1 (0.8–1.4)
rs10860862	**C**/T	3’ UTR	0.16	0.70	0.28	0.02	0.47	1.0	0.9 (0.8–1.1)	0.9 (0.5–1.6)
IGF-2										
rs734351	**C**/T	intron	0.22	0.56	0.38	0.06	0.70	1.0	1.1 (0.9–1.3)	1.1 (0.8–1.6)
rs3802971	**C**/T	3’ UTR	0.17	0.69	0.28	0.03	0.51	1.0	1.0 (0.8–1.2)	1.4 (1.5–1.2)
rs2585	**T**/C	3’ UTR	0.44	0.33	0.46	0.21	0.25	1.0	1.0 (0.8–1.2)	1.2 (0.9–1.5)

* Major allele is in bold

† Minor allele frequency (MAF) based on 1,110 cases and 1,203 controls

[≠] For SNP, AA, major allele homozygote, AB, heterozygote, BB, minor allele homozygote, among controls

[§] P-value is the probability of the Chi-square test for Hardy Weinberg disequilibrium among controls

[‖] Logistic regression models conditioned on age, and adjusted for menopausal status, age at menarche, and age at first full term pregnancy.

Table 2

Association between *IGF-1* and *IGF-2* haplotypes and breast cancer risk.

Haplotype	Frequency		Odds ratio (95% CI)*
	Cases (*n=1,055*)	Controls (*n=1,059*)	
IGF-1			
block 1†			
TCCA	38.1	37.8	1.0
CCCA	15.8	16.1	1.0 (0.8–1.2)
CCTC	28.1	27.9	1.0 (0.9–1.2)
CTTA	16.5	16.7	0.9 (0.7–1.1)
block 2‡			
TT	56.6	55.9	1.0
TC	18.0	18.0	1.0 (0.8–1.2)
CC	25.2	25.6	1.0 (0.8–1.1)
block 3§			
CG	26.2	27.4	1.0
CC	58.2	56.7	0.8 (0.5–1.4)
TG	15.6	15.9	1.0 (0.5–2.1)
block 4‖			
AC	66.1	64.3	1.0
AT	5.9	6.0	1.0 (0.7–1.3)
TT	27.5	28.5	0.9 (0.8–1.1)
IGF-2			
Block 1**			
TCC	55.2	57.3	1.0
CCT	24.2	23.3	1.1 (0.8–1.7)
CTC	16.7	15.8	1.1 (0.9–1.3)

* Additive model, adjusted for age, menopausal status, age at menarche, and age at first full term pregnancy

† *rs10860861*, *rs6219*, *rs2946834*, and *rs5742726*

‡ *rs6218* and *rs6220*

§ *rs4764697* and *rs2195239*

‖ *rs2288377* and *rs35767*

** *rs2558*, *rs3802971*, and *rs734351*