Null Results in Brief

The A^{-336}C Insulin-Like Growth Factor Binding Protein-3 Promoter Polymorphism Is Not a Modulator of Breast Cancer Risk in Caucasian Women

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Introduction

Insulin-like growth factors (IGF) play an important role in the proliferation and apoptosis of several cell types and may therefore be associated with the risk of malignant transformation (1). IGF binding protein-3 (IGFBP-3) is the most common binding protein for IGF-I in serum. High levels of IGFBP-3 are associated with reduced IGF-I levels and thereby influence cell proliferation by modulating access of IGFs to the IGF receptors. In healthy women, alcohol consumption was found to suppress IGF-I and IGFBP-3 levels, and the use of oral contraceptives reduced IGF-I levels and increased IGFBP-3 levels (2). There is increasing evidence from prospective cohort as well as case-control studies that increased serum IGF-I levels are associated with an increased breast cancer risk among premenopausal women (3). Serum IGFBP-3 levels have not consistently been found to be related to breast cancer risk.

Recently, an A → C polymorphism in the *IGFBP-3* promoter region was identified that is related to circulating IGFBP-3 levels (4). The AA genotype was associated with higher circulating IGFBP-3 levels than the AC or CC genotype in men and in premenopausal women (4, 2). The genotype-phenotype correlation was modified by body mass index and height (4). Further, it has been shown in *in vitro* tests that the A allele has higher promoter activity than the C allele (4).

Hypothesis. We hypothesized that the C allele of *IGFBP-3* polymorphism leading to decreased serum IGFBP-3 levels and thereby increased serum IGF-I levels would be associated with increased breast cancer risk. We used a case-control study to examine this *IGFBP-3* polymorphism as a potential risk factor for breast cancer risk in premenopausal Caucasian women. We also investigated whether other factors, such as alcohol consumption, use of oral contraceptives, and body size, modify the association.

Materials and Methods

Study Population. The population-based breast cancer case-control study in Germany (1992-1995) enrolled 603 breast cancer patients and 1,068 age-matched controls. All patients were ages <51 years at the time of diagnosis of incident *in situ* or invasive breast cancer. A group of 476 cases and 866 controls were premenopausal, and 34 cases and 65 controls were postmenopausal. The women who had undergone hysterectomy but not bilateral ovariectomy were considered to be of unknown menopausal status (93 cases and 137 controls; for details, see ref. 5). All participants provided a blood sample and completed a self-administered questionnaire eliciting information on known and suspected risk factors for breast cancer.

***IGFBP-3* Sequence.** We compared the *IGFBP-3* promoter polymorphism sequence referred previously to ref. 4 (accession no. M 35878) with sequences available from genome databases (AC 091524 and AX 323409) and three sequences from our own samples (DNA I-III, sequences obtained from GENterprise, Mainz, Germany). The multiple sequence alignment showed differences between the *IGFBP-3* sequences: (*a*) At positions 40,449 and 40,448 of AC 091524 as well as on the same sections of DNA I to III, we found two cytosine bases missing on M 35878 and AX 323409. (*b*) At positions 40,344 and 40,239 of AC 091524 as well as on the equivalent positions of DNA I to III, we found one cytosine base each, which was not present on M 35878 and AX 323409. (*c*) At positions 40,253 and 40,252 of AC 091524, we found one guanine base followed by one cytosine base and the same was true for DNA I to III, whereas on M 35878 and AX 323409, the order was inverse (i.e., CG). The *IGFBP-3* polymorphism analyzed is therefore in position −204 (not −202 as described in ref. 4) relative to the CAP site and at position −336 relative to the ATG start codon). Genotyping was done based on PCR followed by RFLP (4).

Statistical Analysis. We used the χ^2 test to assess departures of the genotype distribution from Hardy-Weinberg equilibrium. For the assessment of *IGFBP-3* genotypes in association with breast cancer risk, we used multivariate logistic regression (SAS Institute, Cary, NC) to calculate odds ratios and 95% confidence intervals (95% CI). Women with AA genotype were taken as reference group. The analyses were stratified by age in 5-year intervals and adjusted for family history of breast cancer in first-degree relatives (yes, no), duration of breast-feeding (continuous), number of full-term pregnancies (1-2, >3), daily amount of alcohol (1-5, 6-11, 12-18, >19 g), use of oral contraceptives (yes, no), and menopausal status (premenopausal, postmenopausal, unknown).

Results

The A allele frequency was 0.48 in cases and 0.47 in controls, and the C allele was 0.52 in cases and 0.53 in controls. AC genotype was the most frequent genotype, being 49.0% in both cases and controls (Table 1). The genotype frequencies were in Hardy-Weinberg equilibrium. There were no significant differences between cases and controls in the frequencies of the *IGFBP-3* polymorphism alleles A and C and the genotypes AA, AC, and CC. Compared with the AA genotype, the adjusted odds ratio (95% CI) was 0.99 (0.77-1.27) and 0.97 (0.73-1.29) associated with the AC and CC genotypes, respectively. Further adjustment for

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Table 1 Genotype frequency of *IGFBP-3*$^{-336}$ polymorphism in cases and controls

	Cases (n = 603), n (%)	Controls (n = 1,068), n (%)	Odds ratio (95% CI)	Odds ratio* (95% CI)
Genotype				
AA	141 (23.4)	243 (22.8)	1.0	1.0
AC	298 (49.4)	521 (48.8)	0.98 (0.76-1.26)	0.97 (0.75-1.26)
CC	164 (27.2)	304 (28.5)	0.93 (0.71-1.23)	0.94 (0.70-1.25)
Premenopausal				
AA	112 (23.5)	189 (21.8)	1.0	1.0
AC	236 (49.6)	429 (49.5)	0.92 (0.69-1.22)	0.91 (0.68-1.22)
CC	128 (26.9)	248 (28.6)	0.86 (0.63-1.18)	0.86 (0.62-1.19)
Postmenopausal				
AA	10 (29.4)	17 (26.2)	1.0	1.0
AC	17 (50.0)	29 (44.6)	1.00 (0.37-2.72)	0.83 (0.25-2.75)
CC	7 (20.6)	19 (29.2)	0.66 (0.21-2.11)	0.67 (0.17-2.64)
Unknown status				
AA	19 (20.4)	37 (27.0)	1.0	1.0
AC	45 (48.4)	63 (46.0)	1.38 (0.70-2.70)	1.32 (0.64-2.69)
CC	29 (31.2)	37 (27.0)	1.59 (0.76-3.33)	1.71 (0.77-3.75)

*Adjusted for family history of breast cancer, duration of breast-feeding, number of full-term pregnancies, daily amount of alcohol/gram, use of oral contraceptives, and, where appropriate, menopausal status.

established risk factors did not alter these estimates. Point estimates differed somewhat by menopausal status; however, 95% CIs were wide. We also failed to detect any association between *IGFBP-3* polymorphism and breast cancer risk in subgroups of women differentiated by alcohol consumption, use of oral contraceptives, and body mass index.

Conclusions

Using a population-based, case-control study of breast cancer by age 50 years, we were not able to find an association between *IGFBP-3* promoter polymorphism and breast cancer risk. Our study confirms the recent results of Schernhammer et al. (6), indicating that the A^{-336}C *IGFBP-3* polymorphism alone does not play an important role in the etiology of breast cancer.

Further work should consider that the circulating IGFBP-3 levels might be strongly influenced by exogenous factors (environmental factors or hormones) other than the genotype. Simultaneous consideration of variability at several polymorphic sites in both *IGFBP-3* and *IGF-I* (2, 7) may also be necessary to gain more information about the associations of the IGF systems with breast cancer.

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