A SEQUENCE REPEAT IN THE INSULIN-LIKE GROWTH FACTOR-1 GENE AND RISK OF BREAST CANCER

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Insulin-like growth factor-1 (IGF-I), a potent mitogen, is hypothesized to influence breast cancer risk. In 3 previous studies, a polymorphism in the *IGF-1* gene (sequence repeat length) was associated with plasma IGF-I level. We evaluated prospectively the relationships among a $(CA)_n$ repeat polymorphism in the *IGF-1* gene, IGF-I level and breast cancer risk in a nested case-control study conducted within the Nurses' Health Study. Blood samples were collected in 1989–1990; up to June 1994, we identified 463 cases of breast cancer. One to 2 controls were selected per case, matched by age, menopausal status, postmenopausal hormone use, month and time of day of blood collection and fasting status, for a total of 622 controls. Although no significant trend was observed, plasma IGF-I levels were significantly lower among controls, with no copy of the *19* allele, compared with those homozygous for the *19* $(CA)_n$ repeat length (146 and 173 ng/ml, respectively; *p*-value for pairwise mean comparison = 0.005). In conditional logistic regression, controlling for established breast cancer risk factors, we observed no significant association between $(CA)_n$ repeat length genotype and risk of breast cancer [compared with repeat genotype *19/19*—*18/19* genotype relative risk (RR) = 0.96, 95% confidence interval (CI) = 0.56–1.64; *18/20* genotype RR = 0.92, 95% CI = 0.39–2.19; *19/20* genotype RR = 1.16, 95% CI = 0.82–1.64; *19/21* genotype RR = 0.69, 95% CI = 0.42–1.14; *20/20* genotype RR = 0.55, 95% CI = 0.28–1.10; *20/21* genotype RR = 0.72, 95% CI = 0.29–1.79]. Results did not vary substantially when evaluated according to menopausal status, tumor receptor status or category of other breast cancer risk factors. Although a modest association cannot be excluded, our data do not support an important relation between this *IGF-1* gene polymorphism and breast cancer risk.

Key words: *insulin-like growth factor-1 polymorphism; breast cancer; epidemiology*

Insulin-like growth factor-1 (IGF-I) is a peptide growth hormone that promotes the proliferation of normal and malignant breast epithelial cells, whereas the principle IGF binding protein, IGFBP-3, slows breast cell proliferation and promotes apoptosis.[1–8] Circulating concentrations of IGF-I and IGFBP-3 vary substantially between individuals.[9,10] Recent epidemiologic studies have suggested that, among premenopausal women, plasma levels of IGF-I are directly associated and those of IGFBP-3 are inversely associated with subsequent risk of breast cancer.[11–14]

Twin studies have indicated that about 38% of the between-person variation in circulating IGF-I levels is genetically determined.[15] Identification of the genetic factors that regulate plasma and tissue IGF-I levels may increase our understanding of the risk of breast cancer in women. A highly polymorphic cytosine-adenosine $(CA)_n$ dinucleotide repeat 1 kb upstream from the transcription start site of *IGF-1* has been identified.[16,17] This polymorphism lies within the promoter region of *IGF-1* that has been shown in rats to contain regulatory elements that may alter transcription.[18,19]

To date, the relation between this *IGF-1* polymorphism and plasma IGF-I levels has been evaluated in 3 studies. In 2, the most common genotype for this polymorphism—homozygosity of *19* $(CA)_n$ repeats—was found to be associated with lower plasma IGF-I levels.[20,21] In the third, homozygosity of *19* $(CA)_n$ repeats was found to be associated with higher IGF-I levels.[22]

To explore the relations with IGF-I level and breast cancer risk, we conducted a nested case-control study within the large, prospective Nurses' Health Study cohort. We evaluated the relationships overall and also assessed whether the associations were modified by other breast cancer risk factors.

MATERIAL AND METHODS

Study population

The Nurses' Health Study cohort was established in 1976 when 121,700 female registered nurses 30–55 years of age completed and returned a mailed questionnaire. The cohort continues to be followed every 2 years by questionnaire to update exposure status and to identify cases of newly diagnosed disease. Data have been collected on most breast cancer risk factors including height, weight, age at menarche and menopause, age at first birth, postmenopausal hormone use and family history of breast cancer.

In 1989–1990, blood samples were collected from 32,826 cohort members who were 43–69 years of age at blood collection. Details regarding the blood collection methods have been previously published.[23] Briefly, each woman arranged to have her blood drawn and then shipped, via overnight courier and with an ice-pack to keep the sample cool, to our laboratory where it was processed and separated into plasma, red blood cell and white blood cell components. Samples have been archived in continuously monitored liquid nitrogen freezers since collection. As of 1994, the follow-up rate among the women who provided blood samples was 98%.

Cases in this analysis are women with no reported cancer diagnosis (other than nonmelanoma skin cancer) prior to blood collection and who were diagnosed with breast cancer after blood collection but before June 1, 1994. Overall, 470 cases of breast cancer (395 invasive, 67 in situ, 8 level of invasion unknown) were reported. All cases were confirmed by medical record review, with one exception in which the nurse confirmed the diagnosis but the medical record was unavailable; because of the high confirmation rate upon medical record review (99%), this case was kept in the analysis. Time from blood collection to diagnosis ranged from less than 1 month to 57 months [mean (standard deviation) = 28 (16) months]. Controls were randomly selected participants with no previous cancer report (except nonmelanoma skin cancer) up to and including the interval in which the case was diagnosed. One control was matched per case by year of birth, menopausal status (postmenopausal versus not), recent postmenopausal hormone (PMH) use defined as use within 3 months of blood collection versus not, month of blood collection, time of day of blood draw and fasting status ($\geq$10 hr since a meal versus not). For each of the 150 postmenopausal cases not using PMH at blood collection, a second control was selected to increase the statistical power in analyses of plasma steroid hormones.[23] The study was approved by the Committee on the Use of Human Subjects in Research at the Brigham and Women's Hospital.

We evaluated IGF-1 genotype and breast cancer risk both overall and by menopausal status. Women were defined as premenopausal if they reported having at least 1 natural menstrual cycle in the previous 12 months or if they reported a surgical menopause without bilateral oophorectomy and were under 48 years (if a nonsmoker) or under 46 years (if a current smoker)—ages when less than 10% of the cohort had gone through natural menopause. Women were defined as postmenopausal if they reported having a natural menopause or a bilateral oophorectomy, or, for women who reported a hysterectomy with either 1 or both ovaries remaining, when they were 56 years (if a nonsmoker) or 54 years (if a current smoker)—ages when natural menopause had occurred in 90% of the cohort. Women with uncertain menopausal status were excluded from menopause-specific analyses. Estrogen and progesterone receptor status of the breast tumors was extracted from the medical record.

Laboratory analyses

IGF-I genotyping. Genomic DNA was extracted from buffy coats using the Qiagen Qiamp Blood Kit (Qiagen, Chatsworth CA). The oligonucleotide primers used for PCR were as follows: 5′-GCTAGCCAGCTGGTGTTATT-3′ and 5′-ACCACTCTGG-GAGAAGGGTA-3′. The forward primer was 5′-labeled with a fluorescent dye for automated fragment analysis. Forty nanograms of genomic DNA was used per 25 μl reaction with 30 pmol of each primer, 0.2 μM dNTPs, 1.5 mM MgCl$_2$, 10× PCR Buffer (PE, Foster City, CA) and 1.25 U Taq polymerase (Amplitaq; PE). Amplification conditions were 35 cycles of 94°C for 30 sec, 61°C for 30 sec and 72°C for 30 sec. Rapid fragment length detection was performed using the ABI 377 DNA Sequencer (PE). Representative homozygotes (19/19 and 20/20) were sequenced to confirm (CA)$_n$ repeat number. Amplified products were determined relative to Gene Scan-500 size standard using Genescan and Genotyper Analysis software (PE). Genotyping was performed by laboratory personnel blinded to case-control status, and blinded quality control samples were inserted to validate genotype identification procedures; concordance for the blinded sample was 100%.

Hormone analyses. IGF-I and IGFBP-3 were assayed by ELISA with reagents from Diagnostic Systems Laboratory (Webster, TX). Masked split specimens included within each batch were used to calculate the coefficient of variation within and between batches. For IGF-I and IGFBP-3, these were 8.7 and 9.3%, respectively. Details of the assay methods have been published previously.[3]

Covariate data

Information on other breast cancer risk factors was obtained from 1 or more of the biennial NHS questionnaires. Age at menarche and height were queried in 1976. Age at first birth and parity were asked in 1976 and updated until 1982. Physical activity was obtained from the 1986 NHS questionnaire. The variable used in this analysis is based on the time spent per week in moderate or vigorous recreational activity (including walking, jogging, running, biking, swimming, aerobics, racquetball and tennis). Family history of breast cancer was asked in 1976 and updated in 1982 and 1988. History of benign breast disease was asked every 2 years and updated through 1988 for this analysis. Oral contraceptive use was asked every 2 years through 1984. Weight at age 18 was asked in 1980; current weight was obtained from the questionnaire completed at blood collection. Postmenopausal status and PMH use was asked every 2 years and was updated until diagnosis of breast cancer, when cases were identified and matched to controls.

Data analysis

Women with an IGF-1 sequence repeat genotype of 19/19 were used as the referent group in all analyses. In the main analyses, women were categorized by their repeat length genotype. If a genotype occurred in less than 2% of the participants, it was included in an "other" exposure category. We therefore established a total of 8 genotype categories (18/19, 18/20, 19/19, 19/20, 19/21, 20/20, 20/21, other). For subanalyses when this detailed categorization was not possible, we dichotomized the repeat genotypes as 19/19 versus other, as done in the 2 earlier publications that demonstrated an association with IGF-I level.[20,21] Because this strategy combined genotypes with alleles that are both shorter and longer than the (CA)$_{19}$, we also conducted analyses restricted to 19/19 versus alleles longer than 19/19. We observed little difference between this truncated dichotomization compared with the more inclusive "19/19 versus other" dichotomization and therefore do not report these latter results.

For analyses of the relation with IGF-I and IGFBP-3 levels, in addition to primary analyses with the 8 genotype categories, we trichotomized the repeat length exposure as homozygous for the 19 CA repeat length, heterozygous for the 19 allele and no copies of the 19 allele, as was done in a recent publication.[22] Tests for trend in the relation between CA repeat length and plasma IGF-I and IGFBP-3 levels were conducted by modeling IGF-I genotype as an ordered categorical variable and calculating a Wald statistic. All reported p-values are based on 2-sided tests.

Plasma hormone levels were analyzed using the subset of control women who were not postmenopausal hormone users at blood collection, because current PMH use substantially decreases circulating IGF-I levels.[24] To assess whether plasma IGF-I or IGFBP-3 levels varied by IGF-1 polymorphism length, we used generalized linear models controlling for the matching variables. IGF-I levels were first log-transformed to improve normality.

We used conditional logistic regression to estimate relative risks (odds ratios) and 95% confidence intervals in the total data set. To increase statistical power, we used unconditional logistic regression controlling for the matching factors for all subset analyses (e.g., analyses according to menopausal status, by hormone receptor status and when addressing potential gene-environment interactions). These subset analyses were also conducted using conditional logistic regression, and the results were similar although less precise.

The interaction between this IGF-I repeat polymorphism and established breast cancer risk factors was evaluated by adding indicator terms for the cross-classified variables (e.g., IGF-I genotype (dichotomized as 19/19 versus other) × body mass index (dichotomized at the median among the controls) to the logistic models; the presence of an interaction was assessed using the likelihood ratio test and comparing the model with both the main effects and the interaction terms to that with the main effects only. These analyses were conducted among all women combined.

Seven cases and 22 controls did not have DNA available for genotyping or could not be genotyped; hence 463 cases and 622 controls are included in this analysis. In all, 65 cases (14%) were premenopausal at diagnosis, 356 (77%) were postmenopausal and the remainder (9%) were of unknown menopausal status. The women ranged in age from 43 to 69 years [mean = 58; standard deviation (SD) = 7.1]. Only 8 (0.8%) of the women were either African-American, Asian or Hispanic. Additional descriptive characteristics for this data set have been previously published.[23,25] Among controls, *IGF-1* genotype frequency was similar to that previously reported among Caucasian populations[20,21]; 40% were *19/19*, 23% *19/20*, 11% *19/21* and <10% were any other polymorphism length. These prevalences were similar for cases (see Table II).

Among the controls who had plasma hormone data available and were not using PMHs at blood collection, we assessed whether plasma IGF-I or IGFBP-3 levels varied according to *IGF-1* genotype (Table I). In pairwise comparisons, neither IGF-I nor IGFBP-3 differed significantly by *IGF-1* genotype. We observed a weak trend of decreasing IGF-I level with increasing $(CA)_n$ repeat length genotype, although this was not statistically significant (p-value, test for trend = 0.08). The geometric mean IGF-I levels ranged from 149 ng/ml among those with *IGF-1* genotype *20/20* to 179 among those with *IGF-1* genotype *18/19*. No relation was observed with IGFBP-3 level after controlling for IGF-I level (p-value, test for trend = 0.51). When analyses were restricted to premenopausal controls (n = 70), neither IGF-I nor IGFBP-3 differed significantly by *IGF-1* genotype, although the trends were in the same direction as they were for all controls (p-value, IGF-I test for trend = 0.06; p-value, IGFBP-3 test for trend = 0.06; data not shown). Results among breast cancer cases only were quantitatively similar but not statistically significant (data not shown).

We also stratified analyses of the relation between plasma levels of IGF-I and IGFBP-3 and *IGF-1* genotype by the matching variables (fasting for 10+ *versus* <10 hours before blood draw, blood drawn in the morning *versus* after noon and past *versus* never use of PMHs; data not shown). No significant trend in IGFBP-3 level across *IGF-1* genotype was observed in any strata. Although associations with IGF-I tended to be stronger among those who were fasting at the time of blood draw, among those whose blood was drawn in the morning, and among those who were never PMH users, none of the tests for heterogeneity were statistically significant.

When we collapsed *IGF-1* genotype into 3 groups (*19/19*, 1 copy of genotype *19*, no copies of genotype *19*) as published by Vaessen *et al.*,[22] there was no significant difference between the geometric mean of the plasma IGF-I level among those controls homozygous and those heterozygous for the *19 (CA)_n* repeat length genotype (p-value = 0.78). However, we observed a significantly higher plasma IGF-I level among those with 2 copies of the *19 (CA)_n* repeat genotype (geometric mean = 173 ng/ml) compared with those with no copies of the *19* allele (geometric mean = 146 ng/ml, p-value for the pairwise mean comparison = 0.005). When analyses were restricted to premenopausal controls, the differences in mean IGF-I were attenuated (*19/19* geometric mean = 193

ng/ml, 1 copy of genotype *19* = 175 ng/ml, no copies of genotype *19* = 155 ng/ml; p-value for the pairwise mean comparison of *19/19* and no copies of genotype *19* = 0.10). In addition, we did not observe a significant association between mean height and the number of *19* alleles (p-value, test for trend = 0.47; data not shown).

We did not observe a significant association between *IGF-1* genotype and breast cancer risk, when these factors were assessed overall or among invasive cases only (Table II). Controlling for other breast cancer risk factors (age at menarche, age at menopause, parity, age at first birth, family history of breast cancer, body mass index at age 18, weight change since age 18 and personal history of benign breast disease—see footnote to Table II for categories used) did not alter any of the estimates substantially. The association between IGF-I genotype and breast cancer risk did not vary by menopausal status (Table II).

When we evaluated the relations among subsets of cases defined by their estrogen (ER) and progesterone (PR) receptor status, a slightly decreased risk of breast cancer was observed among those with an *IGF-1* genotype other than *19/19* among the ER−/PR− cases, although the associations were not statistically significant (Table III).

We investigated whether any of these relationships varied according to status of other breast cancer risk factors. We observed no evidence of effect modification by body mass index [likelihood ratio test (LRT), p-value = 0.49], physical activity (LRT, p-value = 0.69), history of benign breast disease (LRT, p-value = 0.42), oral contraceptive use (LRT, p-value = 0.49) or ever use of PMHs (LRT, p-value = 0.27). Among women who had never used PMHs, the relative risk for having genotypes other than *19/19* was 0.76 (95% CI = 0.51–1.14), whereas among women who had ever used PMHs, the comparable relative risk was 1.19 (95% CI = 0.84–1.68). We did not observe a significant interaction between *IGF-1* genotype and family history of breast cancer (LRT, p-value = 0.17). Among women with no family history, the relative risk for *19* homozygotes was 1.05 (95% CI = 0.80–1.38), whereas among women with a family history, the comparable relative risk was 0.49 (95% CI = 0.20–1.23).

DISCUSSION

We did not observe a significant relation between this *IGF-1* $(CA)_n$ repeat polymorphism and risk of breast cancer, either overall or according to menopausal or tumor receptor status. The association did not vary substantially within strata of other known breast cancer risk factors. Although we did not observe a trend in IGF-I level with increasing number of $(CA)_n$ repeats, we did observe significantly higher IGF-I levels in those homozygous for the *19* allele compared with those with no copy of the *19* allele. Strengths of this study include its size and the prospectively collected environmental, reproductive and biomarker data, reducing concerns of recall bias or blood sample timing relative to breast cancer diagnosis.

The relation between plasma IGF-I and breast cancer risk has been evaluated in several recent epidemiologic studies. A positive relation between plasma IGF-I levels and breast cancer risk was

TABLE I – GEOMETRIC MEAN LEVELS OF PLASMA INSULIN-LIKE GROWTH FACTOR (IGF-I) AND IGF BINDING PROTEIN 3 (IGFBP-3) BY *IGF-1* GENOTYPE[1]

	IGF-1 genotype					
	18/19	19/19	19/20	19/21	20/20	Other
No. (%)	41 (9.8)	164 (39.2)	95 (22.7)	41 (9.8)	23 (5.5)	54 (12.9)
IGF-I (ng/ml)	179	173	169	166	149	153
IGFBP-3 (ng/ml)	4200	4246	4150	3997	4074	3898

[1]Adjusted for age, date of blood draw, time of blood draw and fasting status and assessed only among controls who were not current users of postmenopausal hormones. p-values for pairwise mean comparisons (not including comparison with "other") were all >0.08 for IGF-I and >0.14 for IGFBP-3. p-values for tests of trend (not including "other") were: IGF-I, p-trend = 0.08; IGFBP-3, p-trend = 0.15.

TABLE II – RELATIVE RISK (RR) OF BREAST CANCER ACCORDING TO INSULIN-LIKE GROWTH FACTOR I (*IGF-I*) GENOTYPE[1]

	IGF-I genotype							
	18/19	18/20	19/19	19/20	19/21	20/20	20/21	Other[2]
All women (n = 463 cases, 622 controls)								
Cases [no. (%)]	38 (8.2)	13 (2.8)	185 (40.0)	118 (25.5)	42 (9.1)	15 (3.2)	11 (2.4)	41 (8.9)
Controls [no. (%)]	50 (8.0)	16 (2.6)	251 (40.4)	142 (22.8)	65 (10.5)	32 (5.1)	18 (2.9)	48 (7.7)
Simple RR	1.02	1.16	1.0	1.11	0.84	0.66	0.74	1.11
MV RR[3]	0.96	0.92	1.0	1.16	0.69	0.55	0.72	0.96
MV 95% CI	(0.56–1.64)	(0.39–2.19)	—	(0.82–1.64)	(0.42–1.14)	(0.28–1.10)	(0.29–1.79)	(0.59–1.56)
Invasive cases only (n = 387 cases, 540 controls)								
MV RR	0.92	0.74	1.0	1.07	0.71	0.62	0.84	1.05
MV 95% CI	(0.56–1.53)	(0.32–1.74)	—	(0.75–1.53)	(0.43–1.17)	(0.31–1.24)	(0.36–1.94)	(0.63–1.75)
Premenopausal women (n = 65 cases, 70 controls)								
Simple RR	0.83	—	1.0	0.98	1.43	—	—	0.88
MV RR[4]	0.52	—	1.0	0.87	1.96	—	—	0.57
MV 95% CI	(0.14–1.89)	—	—	(0.32–2.39)	(0.40–9.45)	—	—	(0.16–2.03)
Postmenopausal women (n = 356 cases, 503 controls)								
Simple RR	1.0	1.13	1.0	1.10	0.95	0.79	0.85	1.20
MV RR[4,5]	0.88	1.01	1.0	1.11	0.81	0.71	0.83	1.07
MV 95% CI	(0.50–1.55)	(0.41–2.47)	—	(0.77–1.61)	(0.48–1.36)	(0.35–1.45)	(0.34–2.03)	(0.61–1.87)

[1]Conditional logistic regression models were used in analyses among all women. Unconditional logistic regression, controlling for the matching factors (age, month and time of day of blood collection, fasting status) was used in all other analyses.–[2]Genotype (cases, controls): 10/19 (0,2); 11/16 (0,1); 11/19 (3,3); 11/20 (1,1); 13/19 (1,1); 16/19 (3,1); 16/20 (1,0); 17/18 (2,0); 17/19 (6,10); 17/20 (4,3); 17/21 (0,2); 17/22 (0,1); 18/18 (1,1); 18/21 (4,2); 19/22 (9,9); 19/23 (1,3); 20/22 (3,5); 21/21 (1,2); 22/22 (1,1).–[3]In the multivariate models (MV), covariates were categorized as follows: age at menarche (<12, 12, 13, ≥14 years), menopausal status, parity and age at first birth (nulliparous, 1–2 children/age at first birth less than 25 years, 1–2 children/age at first birth ≥25 years, >2 children/<25 years, >2 children/≥25 years), family history of breast cancer (in mother and/or sister), body mass index at age 18 (<21, 21–22.9, 23–24.9, ≥25 kg/m^2), weight change from age 18 (<5 kg, 5–20 kg, ≥20 kg), personal history of benign breast disease (yes/no).–[4]Multivariate model does not control for menopausal status.–[5]Multivariate model also controls for age at menopause (continuous).

TABLE III – RELATIVE RISK (RR) OF BREAST CANCER ACCORDING TO INSULIN-LIKE GROWTH FACTOR I (*IGF-I*) GENOTYPE, BY RECEPTOR STATUS[1]

	IGF-I genotype		
	19/19	Not 19/19	Longer than 19/19[2]
ER+ (249 cases)			
MV RR	1.0	0.98	0.94
MV 95% CI	—	(0.71–1.35)	(0.66–1.34)
ER− (58 cases)			
MV RR	1.0	0.73	0.74
MV 95% CI	—	(0.41–1.31)	(0.39–1.40)
PR+ (186 cases)			
MV RR	1.0	1.03	1.04
MV 95% CI	—	(0.72–1.48)	(0.70–1.53)
PR− (110 cases)			
MV RR	1.0	0.77	0.63
MV 95% CI	—	(0.50–1.19)	(0.38–1.04)
ER+/PR+ (179 cases)			
MV RR	1.0	1.07	1.06
MV 95% CI	—	(0.74–1.54)	(0.71–1.57)

[1]Unconditional logistic regression analyses were used in this table. Models controlled for same covariates as in Table II. Please refer to Table II footnotes for description of covariates. ER, estrogen receptor; PR, progesterone receptor–[2]Includes 19/20, 19/21, 20/20 and 20/21.

reported in several[11,13,26,27] but not all[28,29] case-control studies. In the largest study, the relation was strongest among premenopausal women. This finding was confirmed by prospective data from our Nurses' Health Study population, in which no association was found among postmenopausal women, but a 3-fold increase in risk was found among premenopausal women when the highest tertile of IGF-I level was compared with the lowest (RR = 2.9, 95% CI = 1.2–6.9).[12] Similarly, Toniolo and colleagues[14] reported an increased risk of breast cancer among premenopausal women that was particularly evident among premenopausal women who were diagnosed before the age of 50 (RR for highest *versus* lowest quartile of IGF-I level = 2.30, 95% CI = 1.07–4.94). Therefore, the evaluation of a polymorphism in IGF-I that may play a role in transcription and thus potentially affect both circulating and tissue IGF-I levels was of interest. Conceivably this polymorphism, if functional, might serve as a marker of lifelong tissue IGF-I levels.

Three previous studies have evaluated the relation between this (CA)$_n$ repeat length polymorphism and plasma IGF-I levels. We observe a prevalence of the *19/19* genotype similar to that previously reported by Rosen and colleagues[20]: among 171 study subjects, 32% were homozygous for the *19* repeat allele, 28% were homozygous for the *19/20* allele and 5% were homozygous for the *20* repeat genotype. However, our results differ in the relation between mean plasma IGF-I levels and genotype. Overall, these authors found that after adjustment for age and sex, the mean serum IGF-I level was significantly lower among those with the *19/19* genotype compared with those with any other genotype (129 ng/ml *versus* 154 ng/ml, *p*-value = 0.03).

In a study of 503 premenopausal women, Jernstrom and colleagues[21] found that among current oral contraceptive (OC) users, the mean IGF-I level was significantly lower among women with at least 1 copy of the *19* repeat genotype compared with women with no copies (339 ng/ml *versus* 266 ng/ml, *p*-value < 0.01). However, no association was found among women who did not use OCs. We were unable to address this finding, because none of our women were current OC users. However, we did not observe any significant differences between the polymorphism and IGF-I level when we evaluated by current PMH use or by past OC or PMH use. Similarly, we observed no effect modification by past OC use on the association between the *IGF-1* polymorphism and breast cancer risk (LRT, *p*-value = 0.49).

The relations that we observed between this $(CA)_n$ repeat length polymorphism and IGF-I levels are most similar to the findings of Vaessen and colleagues.[22] In a study of 50 subjects per genotype stratum—randomly selected from a population-based sample of 900—46.7% were homozygous for the *19* allele, 41.7% were heterozygous and 11.6% had no copy of the *19* allele. Mean serum IGF-I increased with the number of *19* alleles (*p*-value, test for trend = 0.003). Although we did not observe a significant trend across the full range of observed $(CA)_n$ repeat lengths, those homozygous for the *19* allele had a significantly higher geometric mean IGF-I level compared with those with no copy of the *19* allele. We previously observed that each 1 ng/ml increase in IGF-I corresponds with a 0.67% increase in the risk of breast cancer among premenopausal women (data not shown).[12] Based on the difference in geometric means reported here (27 ng/ml), women with 2 copies of the *19* $(CA)_n$ repeat genotype would have just 1.19 times the risk of breast cancer compared with women with no copies of the *19* allele. These results are difficult to interpret, since it is unclear whether the effect (if any) of the polymorphism is driven by absolute $(CA)_n$ repeat length or by the presence or absence of the *19* allele, the most prevalent allele within the general population. Mean height was also reported to increase significantly with the number of *19* alleles (*p*-value, test for trend = 0.01).[22] We were unable to confirm this association (*p*-value, test for trend = 0.47).

Overall, our data do not support an important role for this *IGF-1* polymorphism in the etiology of breast cancer. However, as this is the first assessment of this association to our knowledge, additional studies are warranted. Identification of other functional variants in genes involved in the synthesis of IGF-I would be of interest to gain a better understanding of the relation between plasma IGF-I levels and breast cancer risk.

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REFERENCES

1. Zapf J, Froesch ER. Insulin-like growth factors/somatomedins: structure, secretion, biological actions, and physiological role. Horm Res 1986;24;121.
2. Arteaga CL, Kitten LJ, Coronado EB, Jacobs S, Kull FC Jr, Allred DC, Osborne CK. Blockade of the type I somatomedin receptor inhibits growth of human breast cancer cells in athymic mice. J Clin Invest 1989;84:1418–23.
3. Ruan W, Newman C, Kleinberg D. Intact and amino-terminally shortened forms of insulin-like growth factor I induce mammary gland differentiation and development. Proc Natl Acad Sci USA 1992;89: 10872–6.
4. Jones JL, Clemmens DR. Insulin-like growth factors and their binding proteins: biological actions. Endocr Rev 1995;16:3–34.
5. Yang XF, Beamer W, Huynh HT, Pollak M. Reduced growth of human breast cancer xenografts in hosts homozygous for the lit mutation. Cancer Res 1996;56:1509–11.
6. Ng ST, Zhou J, Adesanya OO, Wang J, LeRoith D, Bondy CA. Growth hormone treatment induces mammary gland hyperplasia in aging primates. Nat Med 1997;3:1141–4.
7. Rajaram S, Baylink DJ, Mohan S. Insulin-like growth factor-binding proteins in serum and other biological fluids: regulation and functions. Endocr Rev 1997;18:801–31.
8. Pollak M. IGF physiology and cancer risk. Eur J Cancer 2000;36: 1224–8.
9. Juul A, Bang P, Hertel NT, Main K, Dalgaard P, Jorgensen K, Muller J, Hall K, Skakkebaek NE. Serum insulin-like growth factor I in 1030 healthy children, adolescents, and adults: relation to age, sex, stage of puberty, testicular size, and body mass index. J Clin Endocrinol Metab 1994;78:744–52.
10. Juul A, Dalgaard P, Blum WF, Bang P, Hall K, Michaelsen KF, Muller J, Skakkebaek NE. Serum levels of insulin-like growth factor (IGF)-binding protein-3 (IGFBP-3) in healthy infants, children, and adolescents: the relation to IGF-I, IGF-II, IGFBP-I, IGFBP-2, age, sex, body mass index and pubertal maturation. J Clin Endocrinol Metab 1995;80:2534–42.
11. Bruning PF, VanDoorn J, Bonfrer JMG. Insulin-like growth factor binding protein 3 is decreased in early-stage operable premenopausal breast cancer. Int J Cancer 1995;62:266–70.
12. Hankinson SE, Willett WC, Colditz GA, Hunter DJ, Michaud DS, Deroo B, Rosner B, Speizer FE, Pollak M. Circulating concentrations of insulin-like growth factor-1 and risk of breast cancer. Lancet 1998;351:1393–6.
13. Bohlke K, Cramer DW, Trichopoulos D, Mantzoros CS. Insulin-like growth factor-I in relation to premenopausal ductal carcinoma in situ of the breast. Epidemiology 1998;9:570–3.
14. Toniolo P, Bruning PF, Akhmedkhanow A, Bonfrer JMG, Koenig KL, Lukanova A, Shore RE, Zeleniuch-Jacquotte A. Serum insulin-like growth factor–I and breast cancer. Int J Cancer 2000;88:828–32.
15. Harrela M, Koistinen H, Kaprio J, Lehtovirta M, Tuomilehto J Eriksson J, Toivanen L, Koskenvuo M, Leinonen P, Koistinen R, Seppala M. Genetic and environmental components of interindividual variation in circulating levels of IGF-I, IGF-II, IGFBP-1, and IGFBP-3. J Clin Invest 1996;98:2612–5.
16. Rotwein P, Pollock KM, Didier DK, Krivi GG. Organization and sequence of the human *IGF-I* gene. J Biol Chem 1986;261:4828–32.
17. Weber JL, May PE. Abundant class of human DNA polymorphisms which can be typed using the polymerase chain reaction. Am J Hum Genet 1989;44:188–96.
18. West CA, Arnett TR, Farrow SM. Expression of IGF-I mRNA variants in rat bone. Bone 1996;19:41–6.
19. McCarthy TL, Ji C, Shu H, Casinghino S, Crothers K, Rotwein P, Centrella M. 17 β estradiol potentially suppresses cAMP-induced *IGF-I* gene activation in primary rat osteoblasts cultures. J Biol Chem 1997;272:18132–9.
20. Rosen CJ, Kurland ES, Vereault D, Adler RA, Rackoff PJ, Craig WY, Witte S, Rogers J, Bilezikian JP. Association between serum insulin growth factor-1 (IGF-1) and a simple sequence repeat in IGF-1 gene: Implications for genetic studies of bone mineral density. J Clin Endocrinol Metab 1998;83:2286–90.
21. Jernstrom H, Chu W, Vesprini D, Tao Y, Majeed N, Deal C, Pollak M, Narod SA. Genetic factors related to racial variation in plasma levels of insulin-like growth factor-1: implications for pre-menopausal breast cancer risk. Mol Genet Metab 2001;72:144–54.
22. Vaessen N, Heutink P, Janssen JA, Witteman, JCM, Testers L, Hofman A, Lamberts SWJ, Oostra BA, Pols HAP, van Duijn CM. A polymorphism in the gene for IGF-I: functional properties and risk for type 2 diabetes and myocardial infarction. Diabetes 2001;50:637–42.
23. Hankinson SE, Willett WC, Manson JE, Colditz GA, Hunter DJ, Spiegelman D, Barbieri RL, Speizer FE. Plasma sex steroid hormone levels and risk of breast cancer in postmenopausal women. J Natl Cancer Inst 1998;90:1292–9.
24. Campagnoli C, Biglia N, Altare F, Lanza MG, Lesca L, Cantamessa C, Peris C, Fiorucci GC, Sismondi P. Differential effects of oral conjugated estrogens and transdermal estradiol on insulin-like growth factor I, growth hormone and sex hormone binding globulin serum levels. Gynecol Endocrinol 1993;7:251–8.
25. Ishibe N, Hankinson S, Colditz G, Spiegelman D, Willett W, Speizer FE, Kelsey KT, Hunter DJ. Cigarette smoking, cytochrome *P450 1A1* polymorphisms, and breast cancer risk in the Nurses' Health Study. Cancer Res 1998;58:667–71.
26. Peyrat JP, Bonneterre J, Hecquet B, Hondermarck H, Adenis A. Plasma IGF-I concentrations in human breast cancer. Eur J Cancer 1993;29a:492–7.
27. Agurs-Collins T, Adams-Campbell LL, Kim KS, Cullen KJ. Insulin-like growth factor-1 and breast cancer risk in postmenopausal African-American women. Cancer Detect Prev 2000;24:199–206.
28. Del Giudice ME, Fantus IG, Ezzat S, McKeown-Eyssen G, Page D, Goodwin PJ. Insulin and related factors in premenopausal breast cancer risk. Breast Cancer Res Treat 1998;47:111–20.
29. Petridou E, Papadiamantis Y, Markopoulos C, Spanos E, Dessypris N, Trichopoulos D. Leptin and insulin growth factor I in relation to breast cancer. Cancer Causes Control 2000:11:383–8.