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Article

ARTICLE

Inclusion of Plasma Prolactin Levels in Current Risk Prediction Models of Premenopausal and Postmenopausal Breast Cancer

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Abstract

Background: Circulating plasma prolactin is associated with breast cancer risk and may improve our ability to identify high-risk women. Mammographic density is a strong risk factor for breast cancer, but the association with prolactin is unclear. We studied the association between breast cancer, established breast cancer risk factors and plasma prolactin, and improvement of risk prediction by adding prolactin.
Methods: We conducted a nested case-control study including 721 breast cancer patients and 1400 age-matched controls. Plasma prolactin levels were assayed using immunoassay and mammographic density measured by STRATUS. Odds ratios (ORs) were calculated by multivariable adjusted logistic regression, and improvement in the area under the curve for the risk of breast cancer by adding prolactin to established risk models. Statistical tests were two-sided.
Results: In multivariable adjusted analyses, prolactin was associated with risk of premenopausal (OR, top vs bottom quintile = 1.9; 1.88 (95% confidence interval [CI] = 1.08 to 3.26) but not with postmenopausal breast cancer. In postmenopausal cases prolactin increased by 10.6% per cBIRADS category (P_{trend} = .03). In combined analyses of prolactin and mammographic density, ORs for women in the highest vs lowest tertile of both was 3.2 (95% CI = 1.3 to 7.7) for premenopausal women and 2.44 (95% CI = 1.44 to 4.14) for postmenopausal women. Adding prolactin to current risk models improved the area under the curve of the Gail model (+2.4 units, P = .02), Tyrer-Cuzick model (+3.8, P = .02), and the CAD2Y model (+1.7, P = .008) in premenopausal women.
Conclusion: Circulating plasma prolactin and mammographic density appear independently associated with breast cancer risk among premenopausal women, and prolactin may improve risk prediction by current risk models.

Prolactin is a lactogenic hormone produced both by the pituitary gland and locally within the breast tissue (1–3). Prolactin promotes differentiation of epithelial cell and alveoli (4,5). Increased levels of prolactin have been associated with several breast cancer risk factors, such as nulliparity, family history of breast cancer, and mammographic breast density (6–8). Reports on the association between prolactin and risk of breast cancer have been conflicting in both case-control studies (9–15) and prospective studies (16–25) and can differ by the hormone receptor status of the tumors. Small numbers of subjects in most studies may preclude the ability to detect true associations between plasma prolactin and breast cancer risk.

Current risk prediction models use lifestyle factors (26), family history of breast cancer (27), mammographic density (28), mammographic features (29), and genetic determinants (30). Although diverse and inconclusive, three previous studies suggest that combined endogenous hormones may to some extent

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improve risk prediction, particularly for invasive breast cancers in postmenopausal women (31–33).

We examined whether circulating plasma prolactin concentrations were associated with breast cancer risk among pre- and postmenopausal women in the large, prospective KARMA study (34). We analyzed associations of reproductive history, breast cancer risk factors, and mammographic density with prolactin levels. Finally, we assessed whether inclusion of prolactin improved risk prediction of premenopausal and postmenopausal breast cancer.

Materials and Methods

Study Population

We used the KARMA (Karolinska Mammography Project for Risk Prediction for Breast Cancer) study, a population-based prospective cohort study initiated in January 2011 comprising 70 877 women attending mammography screening or clinical mammography in Sweden (34,35). The overarching goal of KARMA is to reduce the incidence and mortality of breast cancer by focusing on individualized prevention and screening. Women completed a comprehensive baseline questionnaire and donated nonfasting EDTA plasma samples of peripheral blood at enrolment (34,35). All blood samples were handled in accordance to a strict 30-hour cold-chain protocol and were processed in the Karolinska Institutet high-throughput biobank. Body mass index (BMI) was self-reported at study entry. All available KARMA participants diagnosed with breast cancer after study entry and the initial blood draw but before August 1, 2015, were included in the study. Two controls were age-matched to each case. The mean time to diagnosis was 12.2 months (13.0) and 12.5 months (13.6) for premenopausal and postmenopausal cases, respectively.

Each study participant signed an informed consent form and accepted linkage to national breast cancer registers. The Stockholm ethical review board approved the study (2010/958-31/1).

Risk Scores and Mammographic Density

We estimated the 2- or 5-year risks of breast cancer using the Gail, Tyrer-Cuzick, and CAD2Y risk scores (26,29,36). None of these risk models use plasma hormones or, with the exception of the CAD2Y risk score, mammographic density. Reproductive history and family history of breast cancer are the major determinants included.

Mammograms were collected at study enrolment (34,35). Full-field digital mammograms from the mediolateral oblique and craniocaudal views of the left and right breasts were used to measure mammographic density using the area-based STRATUS method (29). Breast density was categorized on scale cut-points (2%, 17%, 49%) into four composition groups reflecting the clinically accepted Breast Imaging Reporting and Data System (American College of Radiology, Reston, VA). We named the categorization cBIRADS. The CAD2Y model also includes microcalcifications and masses measured and predicts two-year risk of breast cancer and includes: age, menopausal status, BMI, current use of hormone replacement therapy (HRT), breast cancer in family, percent mammographic density, mammographic density (absolute difference between breasts), microcalcification (absolute difference between breasts), and interaction between mammographic density and masses (29).

Laboratory Assays

Prolactin was measured by quantitative sandwich enzyme immunoassay (R&D Systems, Minneapolis, MN) at the section of Analytical Chemistry and Neurochemistry, Department of Chemistry, Uppsala University, in one batch. The limit of detection was 0.6 ng/mL. The relative SD from blinded replicate samples was less than 6%.

Statistical Analyses

Geometric mean prolactin levels adjusted for age and BMI at blood draw across categories of predictive factors were calculated. Outliers (prolactin levels >150 ng/mL; $n=2$) were excluded. Associations were assessed using linear regression where prolactin levels were log-transformed. We calculated Wald statistics to test for differences between regression coefficients for controls and cases. Associations of mammographic density by cBIRADS with prolactin levels were assessed using linear regression, with stratification by cases-control status. Two-sided P values were calculated using the F test.

Odds ratios (ORs) and 95% confidence intervals (CIs) were determined using unconditional logistic regression, adjusting for matching factor (age at blood draw), comparing prolactin levels by quintiles (cut-points based on levels in the control population) or mammographic density by cBIRADS. Tests for trend were carried out using prolactin and density as continuous variables and calculating the Wald statistic. Crude models were adjusted for matching factor age as well as BMI at blood draw, and multivariable models adjusted for age and BMI at blood draw, previous benign breast disorder (no, yes), breast cancer in family (no, yes), age at menarche, number of births (0, 1, 2, or $\geq$3) and smoking status (never, former, or current smoker) in premenopausal women, with the addition of HRT status (current/former vs never user) in postmenopausal women. Tertiles of prolactin and percent density for combined ORs of breast cancer were determined from the distribution among controls. Tests for trend were based on natural log-transformations of prolactin and percent density. We tested for interaction between tertiles of prolactin and density with a likelihood test comparing a model including the main effects and interactions with a model including only the main effects.

We compared the area under the curve (AUC) for the different risk models before and after adding prolactin by adding a linear term for natural log-transformed prolactin by logistic regression (37). We used stepwise regression (entry $P<.15$ for forward and $P<.2$ for backward step) to identify the subset of variables that were the most predictive of breast cancer risk.

For ORs of breast cancer by levels of prolactin, we also stratified by time between blood draw and diagnosis, ER and progesterone receptor (PR) status, and tumor size and invasiveness. We furthermore conducted analyses of ORs of breast cancer among postmenopausal women restricted to never HRT users. Tests were two-sided and considered statistically significant if P was less than .05. Analyses were conducted using SPSS (version 23; IBM corporation). AUCs were evaluated with R 3.4.1 software.

Results

The final study group included 237 premenopausal cases and 410 premenopausal controls and 484 postmenopausal cases and 990 postmenopausal controls (Table 1). Postmenopausal

Table 1. Baseline characteristics for cases and controls, stratified by menopausal status

	Premenopausal women						Postmenopausal women					
	Cases (n = 237)			Controls (n = 410)			Cases (n = 484)			Controls (n = 990)		
Characteristic	No.*	Mean	SD	No.*	Mean	SD	No. *	Mean	SD	No. *	Mean	SD
Age at blood draw, y	237	46.6	4.4	410	46.7	4.3	484	63.8	6.4	990	64.1	6.6
BMI at study entry, kg/m^2	237	24.8	3.8	409	24.9	4.1	482	26.2	4.3	986	25.6	4.1
Age at menarche, y	233	12.9	1.5	404	13.0	1.4	473	13.1	1.5	965	13.3	1.6
Age at first birth, y	199	28.6	5.0	349	28.6	4.8	417	26.0	5.2	885	25.7	5.0
Age at menopause, y							265	50.3	5.3	506	49.9	5.6
Alcohol consumption, g/wk	233	46.9	50.9	408	46.2	56.2	475	59.3	70.2	983	50.1	59.6
Smoking status, %												
Never smoked	116	50.2		227	55.5		182	38.4		438	44.4	
Past smoker	81	35.1		136	33.3		217	45.8		421	42.7	
Current smoker	34	14.7		46	11.2		75	15.8		128	13.0	
Ever use of HRT	21	9.1		42	10.4		235	49.3		449	45.7	
Number of births, %												
Nulliparous	35	15.0		60	14.7		58	12.2		104	10.5	
1 birth	31	13.2		63	15.4		81	17.1		157	15.9	
2 births	120	51.3		191	46.7		229	48.2		462	46.7	
≥3 births	48	20.5		95	23.2		107	22.5		266	26.9	
History of benign breast disease, %	75	28.2		64	18.6		146	31.3		232	23.8	
Family history of breast cancer, %	56	24.5		59	14.9		118	25.0		169	17.6	
Family history of ovarian cancer, %	11	4.9		14	3.6		22	4.8		41	4.4	
Mammographic breast feature												
Mammographic breast density†, %	232	38.3	22.1	409	30.9	20.3	484	16.7	15.1	982	13.4	13.5
Calcifications, mean No.‡	184	0.4	0.7	406	0.1	0.3	360	0.4	0.6	985	0.2	0.5
Calcifications, difference§	184	0.7	1.0	406	0.1	0.4	360	0.7	1.0	985	0.3	0.7
Masses, mean No.‡	184	0.6	0.7	406	0.5	0.5	360	0.9	0.7	985	0.6	0.6
Masses, difference§	184	0.7	0.9	406	0.7	0.7	360	1.1	0.9	985	0.7	0.8
Risk prediction model												
Prolactin, ng/mL	236	21.1	16.1	408	18.1	11.9	482	13.7	10.8	981	13.5	10.2
Gail 5-year risk score‖	233	1.2	0.4	410	1.1	0.4	476	1.2	0.5	990	1.1	0.4
Tyrer-Cuzick 5-year risk score¶	233	1.6	1.0	410	1.4	0.6	476	2.4	1.4	990	2.0	1.0
cBIRADS#, %												
1	5	2.2		21	5.2		62	13.1		190	19.5	
2	42	18.4		107	26.7		224	47.3		484	49.6	
3	113	49.6		203	50.6		174	36.7		275	28.2	
4	68	29.8		70	17.5		14	3.0		26	2.7	
CAD2Y 2-year risk score**	147	0.6	0.5	405	0.3	0.2	303	0.9	0.5	976	0.6	0.4

*Numbers do not always add up to the total owing to missing values. CAD2Y = computer-aided detection 2-year risk; cBIRADS = computer-generated breast imaging reporting and data system score; HRT = hormone replacement therapy.

†Percentage mammographic density at KARMA study entry measured by STRATUS.

‡Mean number in both breasts for each woman.

§Difference of mean number between the left and right breast for each woman.

‖Gail model included risk factors of age, age at menarche, age at first live birth, number of previous breast biopsies, atypical hyperplasia, and first-degree family history of breast cancer.

¶Tyrer-Cuzick model included risk factors of age, age at menarche, age at first child, menopause, length, weight, HRT, hyperplasia, atypical hyperplasia, lobular cancer in situ, and first-/second-degree family history of breast cancer.

#Computer-generated BI-RADS score based on mammographic density at KARMA study entry.

**CAD2Y risk model included BMI, current use of HRT, breast cancer in family, percent mammographic breast density, mammographic density – absolute difference between breasts, microcalcification – absolute difference between breasts, and interaction between mammographic density and masses.

cases had a higher BMI at study entry and were slightly younger at menarche compared with the controls. For all women, cases had more calcifications and were more likely to have benign breast disease and a family history of breast cancer compared with controls. Postmenopausal cases also had significantly more masses. Mean levels of prolactin were higher in premenopausal, in contrast to postmenopausal, cases than controls. For all women, Gail 5-year risk, Tyrer-Cuzick 5-year risk, CAD2Y 2-year risk, and cBIRADS scores were all higher in cases compared with controls.

Among premenopausal controls, number of pregnancies, number of births, and parity were inversely associated with prolactin levels in multivariable analyses ($P = .002$, $P = .002$, and $P = .001$, respectively; Supplementary Table 1, available online). Parous women had 23% lower prolactin compared with nulliparous women, and prolactin decreased by 5% per additional pregnancy and by 9% per birth. Among premenopausal cases, only number of births was inversely associated with prolactin ($P = .001$), which decreased by 13% per additional birth. Minimum duration of breastfeeding was associated with a 1%

reduction in prolactin by additional month of breastfeeding ($P = .02$), whereas there was no significant association between prolactin and breastfeeding among premenopausal controls. Premenopausal controls without a history of smoking had higher prolactin (geometric mean 16.1) compared with former smokers (mean 14.8) and current smokers (mean 12.9) ($P_{trend} = .009$). The same association was not observed for cases. Other factors described in Supplementary Table 1 (available online) were not associated with prolactin levels among premenopausal controls or cases.

Among postmenopausal women, age was inversely associated with prolactin in both controls (0.6% decrease per increasing year, $P = .03$) and cases (0.8% decrease per increasing year, $P = .04$) (Supplementary Table 2, available online).

Using multivariable adjusted analyses, prolactin was not significantly associated with increasing cBIRADS categories among premenopausal women (Table 2). In contrast, among postmenopausal women, prolactin was associated with an increase of 3.3% ($P_{trend} = .03$) per increasing cBIRADS category, which was only observed among cases (10.6%, $P_{trend} = .03$) but not controls (1.0%).

Prolactin was associated with breast cancer risk in premenopausal women in both crude and multivariable analyses (OR = 1.48, 95% CI = .10 to 2.03, $P_{trend} = .01$). The ORs in the highest quintile compared with the lowest were 1.88 (95% CI = 1.08 to 3.26) (Table 3). The addition of percentage mammographic density did not substantially affect these associations (Table 3). Results were similar for premenopausal cases diagnosed within 2 years of blood draw, ER+ and PR+ cases, tumors less than 20 mm in size, and invasive tumors (all $P < .05$) (Supplementary Table 3, available online). Prolactin was not significantly associated with breast cancer risk among postmenopausal women (Table 3; Supplementary Table 3, available online).

Breast cancer risk was positively associated with percentage mammographic density (OR = 1.36, 95% CI = 1.11 to 1.68, $P_{trend} = .003$). ORs in the highest cBIRADS category (cBIRADS 4) compared with the lowest category (cBIRADS 1) were 6.97 (95% CI = 1.97 to 24.66) among premenopausal women and OR = 2.54 (95% CI = 1.13 to 5.73) among postmenopausal women in multivariable models (Table 4). Adding prolactin to the model did not influence these associations.

In joint exposure analyses of combined effects of percentage mammographic density and total prolactin levels, the OR in the top tertile for both was 3.15 (95% CI = 1.29 to 7.65) among premenopausal women and 2.44 (95% CI = 1.44 to 4.14) in postmenopausal women (Table 5). There was no statistical evidence for interaction between density and prolactin on breast cancer risk.

For premenopausal women, the AUC for the different risk models ranged from 54.7 to 64.6 before adding prolactin (Table 6). The addition of prolactin improved the Gail 5-year model (+2.4 units, $P = .02$), the Tyrer-Cuzick 5-year model (+3.8 units, $P = .02$), and the CAD2Y 2-year model (+1.7 units, $P = .008$). The cBIRADS model was not improved. Using stepwise regression, difference in calcifications (OR = 2.38), percent mammographic density (OR = 1.02), history of benign breast disorder (OR = 1.66), breast cancer in family (OR = 1.70), number of calcifications (OR = 2.01), and age at menarche (OR = 0.87) were selected. The AUC for the complete model was 72.2 (Table 6). Adding prolactin to the stepwise selected model improved the AUC by 0.9 units ($P = .03$). The results were similar for ER-positive tumours (data not shown).

For postmenopausal women, the AUC for the different risk models ranged from 55.4 to 68.8 before adding prolactin (Table 6). No improvement was seen by adding prolactin to the models. Using stepwise regression, difference in calcifications (OR = 1.78), number of masses (OR = 1.73), history of benign breast disorder (OR= 1.53), difference in masses (OR = 1.34), smoking status (OR = 1.34), breast cancer in family (OR = 1.61), percent mammographic density (OR =1.02), BMI (OR = 1.04), and ever use of HRT (OR = 1.24) were selected. The AUC for the complete model was 73.1 (Table 6). Adding prolactin to the stepwise model did not change the AUC. The results were similar for ER-positive tumors and when excluding women undergoing HRT treatment at time of blood draw (data not shown).

Discussion

In this large prospective study, circulating prolactin was associated with increased risk of premenopausal breast cancer that was independent of mammographic density. Inclusion of prolactin into the current risk prediction models somewhat improved breast cancer risk prediction among premenopausal women. We found no associations between prolactin and breast cancer risk among postmenopausal women.

In our study plasma prolactin was positively associated with breast cancer risk among the premenopausal women (Table 3), like some (20,22) but not all previous studies (19,31,38). Although the pattern of ORs across quintiles was not clearly linear, the trend tests were statistically significant, suggesting a moderately increased risk of breast cancer with higher prolactin levels.

The strongest relationships between prolactin and breast cancer were seen for premenopausal women diagnosed within 2 years of blood draw (Supplementary Table 3, available online). Hypothetically, breast tumours secrete prolactin (1,39,40). Tworoger et al. (20) postulated that prolactin could be a marker of both risk and an existing tumor. In stratified analyses by tumor types, we observed a positive association for ER+, but not PR+, tumors. Endogenous prolactin and oestradiol have been shown to act synergistically to increase oestrogen responsiveness and proliferation in breast cancer cells (41).

Among postmenopausal women, we found no significant associations between prolactin and breast cancer risk (Supplementary Table 1, available online), a finding repeatedly described (17–19,31,38). In contrast, the Nurses' Health Study (n = 1445 postmenopausal cases) indicated a modest positive association (relative risk = 1.37, 95% CI = 1.11 to 1.69) between circulating plasma levels of prolactin and breast cancer risk among postmenopausal women (21).

We are the first to our knowledge to publish on the interaction of mammographic density and prolactin levels and found that circulating prolactin and mammographic density were independent risk factors for breast cancer among premenopausal women (Tables 3 and 4). Combining mammographic percent density and total prolactin gave a 3-fold increased risk when contrasting premenopausal women at lowest density and prolactin levels to those at highest levels (Table 5). The associations of prolactin and breast cancer were apparent in all strata of mammographic density (and vice versa) without evidence for interaction or effect modification between these factors.

The only two previous reports of combined effects of density and endogenous androgens and estrogens on breast cancer risk included postmenopausal women and found an over 4-fold increased risk of breast cancer in the highest group of hormones and percentage density (42,43). Association of breast cancer risk, irrespective of prolactin levels, was of

Table 2. Association between prolactin levels (ng/mL) and mammographic density, described using cBIRADS, stratified by menopausal and case-control status

Category	cBIRADS 1	2	3	4	% increase per cBIRADS*	P_{trend}*
Premenopausal women						
No. of cases/controls	5/21	42/107	113/202	67/68		
Prolactin, ng/mL, geometric mean†, all women, multivariable model	13.3	15.3	15.9	16.8	8.2	.12
Prolactin, ng/mL, geometric mean†, controls, multivariable model	12.7	14.9	15.5	15.7	7.5	.28
Prolactin, ng/mL, geometric mean†, cases, multivariable model	19.0	17.1	16.5	17.7	−2.1	.82
Postmenopausal women						
No. of cases/controls	62/189	224/475	172/273	14/25		
Prolactin, ng/mL, geometric mean‡, all women, multivariable model	10.8	11.1	11.9	11.9	3.3	.03
Prolactin, ng/mL, geometric mean‡, controls, multivariable model	11.1	11.0	11.8	11.4	1.0	.31
Prolactin, ng/mL, geometric mean‡, cases, multivariable model	9.7	11.1	11.9	13.1	10.6	.03

*Average percentage change in prolactin level per category increase in mammographic density by computer-generated breast imaging reporting and data system score (cBIRADS). *P* value (two-sided) based on *F*-test with natural log-transformed prolactin (ng/mL), continuous, and cBIRADS as dependent variable. cBIRADS = computer-generated breast imaging reporting and data system score.

†Adjusted for age and body mass index at blood draw, benign breast disorder (no, yes), breast cancer in family (no, yes), smoking status (never, previous, or current smoker), age at menarche, and number of births (0, 1, 2, or ≥3).

‡Adjusted for age and body mass index at blood draw, benign breast disorder (no, yes), breast cancer in family (no, yes), smoking status (never, previous, or current smoker), age at menarche, number of births (0, 1, 2, or ≥3), and ever use of hormone replacement therapy.

Table 3. Multivariable adjusted odds ratios for risk of breast cancer as a function of prolactin levels (ng/mL), given in quintals with and without addition of mammographic density, stratified by menopausal status

Category	No. of cases/controls	Plasma prolactin level (ng/mL), quintiles* 1st OR (95% CI)	2nd OR (95% CI)	3rd OR (95% CI)	4th OR (95% CI)	5th OR (95% CI)
Premenopausal women						
Multivariable†	211/381	1.0	1.24 (0.70 to 2.2)	1.08 (0.60 to 1.94)	1.21 (0.68 to 2.15)	1.88 (1.08 to 3.26)
Multivariable† + Density‡	207/380	1.0	1.26 (0.71 to 2.25)	1.12 (0.62 to 2.03)	1.13 (0.63 to 2.03)	1.77 (1.00 to 3.12)
Postmenopausal women						
Multivariable§	436/904	1.0	1.32 (0.91 to 1.91)	1.30 (0.90 to 1.87)	1.04 (0.71, 1.53)	1.07 (0.73 to 1.57)
Multivariable§ + Density‡	436/896	1.0	1.34 (0.93 to 1.95)	1.27 (0.88 to 1.85)	1.07 (0.72 to 1.57)	1.03 (0.70 to 1.51)

*Premenopausal: Q1: <7.01 ng/mL, Q2: 7.01–9.43, Q3: 9.44–12.42, Q4: 12.4–17.22, Q5: >17.22. Postmenopausal: Q1: <9.27 ng/mL, Q2: 9.28–12.89, Q3: 12.90–17.52, Q4: 17.53–24.33, Q5: >24.33. BMI = body-mass index; CI = confidence interval; OR = odds ratio.

†Adjusted for age and body mass index at blood draw, benign breast disorder (no, yes), breast cancer in family (no, yes), smoking status (never, previous, or current smoker), age at menarche, and number of births (0, 1, 2, or ≥3).

‡Percentage mammographic breast density by STRATUS.

§Adjusted for age and body mass index at blood draw, benign breast disorder (no, yes), breast cancer in family (no, yes), smoking status (never, previous, or current smoker), age at menarche, number of births (0, 1, 2, or ≥3), and ever use of hormone replacement therapy.

Table 4. Risk of breast cancer as a function of mammographic density, measured as cBIRADS, with and without addition of prolactin, stratified by menopausal status

Category	No. of cases/controls	cBIRADS* 1 OR (ref.)	2 OR (95% CI)	3 OR (95% CI)	4 OR (95% CI)
Premenopausal women					
Multivariable†	205/375	1.0	2.27 (0.69 to 7.45)	3.49 (1.05 to 11. 55)	6.97 (1.97 to 24.66)
Multivariable† + Prolactin‡	204/373	1.0	2.13 (0.65 to 7.00)	3.26 (0.98 to 10.83)	6.37 (1.79 to 22.63)
Postmenopausal women					
Multivariable§	435/898	1.0	1.79 (1.24 to 2.58)	2.79 (1.83 to 4.25)	2.54 (1.13 to 5.73)
Multivariable§ + Prolactin‡	433/890	1.0	1.79 (1.24 to 2.58)	2.75 (1.80 to 4.19)	2.51 (1.12 to 5.66)

*computer-generated breast imaging reporting and data system score (cBIRADS) based on scale cut-points of mammographic density (<2%, 2–16%, 17–48%, ≥49%). BMI = body-mass index; CI = confidence interval; OR = odds ratio.

†Adjusted for age and BMI at blood draw, benign breast disorder (no, yes), breast cancer in family (no, yes), smoking status (never, previous, or current smoker), age at menarche, and number of births (0, 1, 2, or ≥3).

‡Prolactin concentration, continuous (ng/mL).

§Adjusted for age and BMI at blood draw, benign breast disorder (no, yes), breast cancer in family (no, yes), smoking status (never, previous, or current smoker), age at menarche, number of births (0, 1, 2, or ≥3), and ever use of hormone replacement therapy.

Table 5. Risk of breast cancer in relation to the combined effect of prolactin levels and mammographic density

	Percentage mammographic density (%), tertiles					
	First		Second		Third	
Category	No. of cases/controls	OR (95% CI)	No. of cases/controls	OR (95% CI)	No. of cases/controls	OR (95% CI)
Premenopausal women*,†						
Prolactin first tertile	13/40	1.0	26/47	1.90 (0.78 to 4.67)	30/49	2.71 (1.06 to 6.50)
Prolactin second tertile	16/52	1.04 (0.41 to 2.65)	22/48	1.37 (0.53 to 3.53)	33/36	3.60 (1.44 to 9.00)
Prolactin third tertile	20/44	1.77 (0.73 to 4.34)	30/42	2.69 (1.11 to 6.54)	41/49	3.15 (1.29 to 7.65)
						$P_{interaction} = .44$‖
Postmenopausal women‡,§						
Prolactin first tertile	41/111	1.0	65/112	2.06 (1.24 to 3.35)	54/103	1.86 (1.07 to 3.25)
Prolactin second tertile	42/110	1.09 (0.64 to 1.86)	59/103	1.78 (1.06 to 3.01)	73/109	2.44 (1.42 to 4.17)
Prolactin third tertile	28/105	0.65 (0.36 to 1.19)	44/107	1.49 (0.87 to 2.57)	76/113	2.44 (1.44 to 4.14)
						$P_{interaction} = .11$‖

*Mammographic density: Q1: <18.87%, Q2: 18.88–40.43, Q3: >40.44. Prolactin: Q1: <11.49 ng/mL, Q2: 11.50–19.46, Q3: >19.47. BMI = body-mass index; CI = confidence interval; OR = odds ratio.

†Adjusted for age and BMI at blood draw, benign breast disorder (no, yes), breast cancer in family (no, yes), smoking status (never, previous, or current smoker), age at menarche, and number of births (0, 1, 2, or ≥3).

‡Mammographic density: Q1: <4.55%, Q2: 4.56–15.58, Q3: >15.59. Prolactin: Q1: <8.65%, Q2: 8.66–13.46, Q3: >13.47.

§Adjusted for age and BMI at blood draw, benign breast disorder (no, yes), breast cancer in family (no, yes), smoking status (never, previous, or current smoker), age at menarche, number of births (0, 1, 2, or ≥3), and ever use of hormone replacement therapy.

‖On the basis of a two-sided log-likelihood ratio test.

Table 6. Area under the curve levels for breast cancer risk models with and without prolactin and stepwise regression models

	Premenopausal women			Postmenopausal women		
Model	AUC	SE	*P*†	AUC	SE	*P*†
Prolactin only*	55.0	2.4	–	53.6	1.6	–
Current risk prediction models						
Gail 5-year risk†	55.9	2.4	–	55.4	1.6	–
Gail 5-year risk† + prolactin	58.3	2.4	.02	55.4	1.6	.69
Tyrer-Cuzick 5-year risk§	54.7	2.5	–	60.4	1.6	–
Tyrer-Cuzick 5-year risk§ + prolactin	58.5	2.4	.02	60.4	1.6	.67
cBIRADS*,‖	61.4	2.3	–	59.8	1.6	–
cBIRADS*,‖ + prolactin	62.3	2.3	.07	59.8	1.6	.98
CAD2Y 2-year risk¶	64.6	2.8	–	68.8	1.7	–
CAD2Y 2-year risk¶ + prolactin	66.3	2.8	.008	68.8	1.7	.72
Stepwise regression models						
Model 1#	72.2	2.4	–	–	–	–
Model 1# + prolactin	73.1	2.4	.03	–	–	–
Model 2**	–	–	–	73.1	1.6	–
Model 2** + prolactin	–	–	–	73.1	1.6	.91

*Adjusted for age and BMI at blood draw. AUC = area under the curve; BBD = benign breast disorder; BMI = body-mass index; CAD2Y = computer-aided detection 2-year risk; cBIRADS = computer-generated breast imaging reporting and data system score; HRT = hormone replacement therapy.

†Two-sided *P* value indicates Wald test for addition of natural log-transformed prolactin (ng/mL) to a model including the baseline risk prediction model, adjusted for age.

†Gail model included risk factors of age, age at menarche, age at first live birth, number of previous breast biopsies, atypical hyperplasia, and first-degree family history of breast cancer.

§Tyrer-Cuzick model included risk factors of age, age at menarche, age at first child, menopause, length, weight, HRT, hyperplasia, atypical hyperplasia, lobular cancer in situ, and first-/second-degree family history of breast cancer. Data coding was done according to the Tyrer-Cuzick protocol.

‖Computer-generated BI-RADS score based on mammographic density at KARMA study entry.

¶CAD2Y risk model included age, menopausal status, BMI, current use of HRT, breast cancer in family, percent mammographic breast density, mammographic density – absolute difference between breasts, microcalcification – absolute difference between breasts, and interaction between mammographic density and masses.

#Model 1 selected in premenopausal women by stepwise logistic regression: breast cancer in family; BBD; age at menarche; per cent density; calcifications, difference between breasts; calcifications, average number.

**Model 2 selected in postmenopausal women by stepwise logistic regression: BMI; breast cancer in family; BBD; per cent density; calcifications, difference between breasts; masses, difference between breasts; masses, average number; ever use of HRT; and smoking status.

similar magnitude in our study to previous studies (42,43). Likewise, associations of risk with prolactin levels, irrespective of density, were of similar albeit lower magnitude in our study compared with the associations of sex hormones previously reported (31,32). The positive association between prolactin and mammographic density among postmenopausal women in our study is supported by some (6,8), but not all (44,45) past studies.

To the best of our knowledge, this is the first study to show a positive association between prolactin and mammographic density among postmenopausal cases but not their matched controls (Table 2). Collectively, our findings suggest that prolactin independently affects density among postmenopausal women.

Inclusion of endogenous prolactin to established risk models improved risk prediction by the Gail, Tyrer-Cuzick, and CAD2Y risk prediction models among premenopausal women, whereas there was no distinguishable change among postmenopausal women (Table 6). A recent study included prolactin and testosterone along with other risk factors in a final model for premenopausal women, but not postmenopausal women, and reached and AUC of 84.2 (33). In our final stepwise regression model for premenopausal women, prolactin was added to history of benign breast disorder, family history of breast cancer, age at menarche, mammographic percentage density, and calcifications and reached an AUC of 73.1 compared with an AUC of 55.9 for Gail alone. Collectively, prolactin may add value to risk prediction among premenopausal women. The additional predictive value may, however, be attenuated because prolactin is correlated with factors included in the risk prediction models such as age, reproductive history, and mammographic density.

Of the three previous studies examining hormones in postmenopausal risk prediction, two did not include prolactin in the final model (31,33), whereas one study included prolactin for ER+ cancers (32). Together with our findings, this suggests that inclusion of prolactin alone for postmenopausal risk prediction does not provide any additional value. Inclusion of biomarkers representing the three hormonal axes estrogens, androgens, and growth hormones are likely needed to see the full biological effect.

Like previous studies, premenopausal parous healthy women had lower prolactin levels than nulliparous women (Supplementary Table 1, available online), with the greatest decrease seen after the first full-term pregnancy (7,46). Similar albeit not significant effects were found among cases. Similar to some (45,46), but not all previous studies (7,47), we found no significant associations between reproductive history and prolactin among postmenopausal women, thus not supporting the hypothesized role of prolactin as a mediator of long-term risk reducing effects by parity.

Premenopausal never and former smokers had higher levels of prolactin than current smokers, with a similar although nonsignificant trend among postmenopausal women. This finding is supported by one previous study in postmenopausal women (45). Among cases, the effects of smoking on prolactin levels were weaker and nonsignificant.

This study has some limitations. First, we used data from a single biomarker measurement collected at study entry up to 5 years prior to diagnosis in these analyses. However, the within-person stability of prolactin over time, up to 10 years prior to diagnosis, has been previously demonstrated for both premenopausal and postmenopausal women (21,48,49). Second, the immunoassay measured multiple prolactin isoforms, which may have different biologic activities (50,51); thus, based on our results we cannot identify which isoforms are most important for breast cancer risk or mammographic density. Third, all exposure data is self-reported, which may result in some misreporting. However, exposure data, mammograms, and blood samples were collected at the same time at KARMA study entry. Furthermore, a nondifferential misclassification of exposures would dilute, not strengthen, the reported associations.

Strengths of our study include that blood samples were collected before disease onset, the large number of samples, the possibility to use four independently validated risk scores on the dataset, and the possibility to match study participants to the national breast cancer register. Furthermore, the KARMA study provides centralized collection and handling of mammograms and blood samples, the quantitative assessment of mammographic density by STRATUS, and collection of background information of all participants (34). This is also, to the best of our knowledge, the first prospective study investigating circulating prolactin in relation to reproductive history and breast cancer risk factors in controls and cases separately.

This prospective study, including 237 premenopausal and 484 postmenopausal breast cancer cases, shows that higher circulating prolactin levels were associated with an 80% higher risk of breast cancer for women in the highest vs the lowest prolactin concentrations among premenopausal women independent of mammographic density. In particular, prolactin was associated with more proximate risk. By contrast, risk was independent of circulating prolactin among postmenopausal women. Inclusion of prolactin to established and independently validated risk scores improved discrimination of premenopausal breast cancers and could potentially add value to targeted screening.

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