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Prolactin receptor expression and breast cancer: relationships with tumor characteristics among pre and postmenopausal women in a population-based case-control study from Poland

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Abstract

Previous studies have found an association between elevated circulating prolactin levels and increased risk of breast cancer. Prolactin stimulates breast cancer cell proliferation, migration, and survival via binding to the cell-surface prolactin receptor. The association of prolactin receptor expression with breast tumorigenesis remains unclear as studies that have focused on this association have had limited sample size and/or information about tumor characteristics. Here we examined the association of prolactin expression with tumor characteristics among 736 cases, from a large population-based case-control study of breast cancer conducted in Poland (2000–2003), with detailed risk factor and pathology data. Tumors were centrally reviewed and prepared as tissue microarrays for immunohistochemical analysis of prolactin receptor expression. Association of prolactin receptor expression across strata of tumor characteristics was evaluated using χ^2 analysis and logistic regression. Prolactin receptor expression did not vary by menopausal status; therefore, data from pre- and postmenopausal women were combined in the analyses. Approximately 83% of breast cancers were categorized as strong prolactin receptor staining. Negative/low prolactin receptor expression was independently associated with poorly differentiated ($p=1.2 \times 10^{-08}$) and larger tumors ($p=0.0005$). These associations were independent of estrogen receptor expression. This is the largest study to date in which the association of prolactin receptor expression with tumor characteristics has been evaluated. These data provide new avenues from which to explore the associations of the prolactin/prolactin receptor signaling network with breast tumorigenesis.

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Keywords

molecular subtypes; prolactin receptor; breast cancer; immunohistochemistry

Background

Prolactin (PRL) and its receptor (PRLR) are necessary for development and differentiation of the normal breast [1–5]. When PRL binds to PRLR, the Jak2/Stat5, PI3K, and MAPK signaling pathways are activated, resulting in enhanced survival, proliferation and differentiation of breast epithelial cells [1, 2, 5]. These signaling pathways lead to expansion of the epithelial cell population in the breast during pregnancy and promote differentiation of the cells that synthesize and secrete milk during lactation.

Evidence from both laboratory and epidemiology studies support a role for PRL and PRLR signaling in breast tumorigenesis. PRL can stimulate cell proliferation, migration, and survival of human breast cancer cells in culture [2, 5–7]. In vivo, high PRL levels are significantly associated with increased breast cancer risk in both large prospective and case-control studies [5, 8–13], independent of established breast cancer risk factors. These results are consistent across both pre- and postmenopausal women. A recent analysis of women from Nurses' Health Study (NHS) I and II found that higher PRL levels from samples collected less than 10 years prior to diagnosis were associated with breast cancer risk, irrespective of menopausal status (relative risk [RR]=1.20, 95% confidence interval [CI]=1.03–1.40) [13]. When this analysis was restricted to only postmenopausal women with estrogen receptor (ER) positive disease, the risk estimate increased to RR=1.52 (95% CI 1.19–1.93) [13]. In a large case-control study, we found that, among postmenopausal women, higher PRL levels were associated with increased risk of breast cancer (OR=1.76, 95% CI 1.21–2.57, $p=0.003$) [12]. Among controls in both studies cited above, circulating PRL levels have been associated with select breast cancer risk factors (e.g. parity, body mass index, menopausal hormone therapy use and mammographic density). In contrast, data relating PRL levels to tumor characteristics are limited and conflicting [5, 8, 10–12].

Epidemiologic studies of PRL and PRLR in association with human breast cancer have predominantly focused on circulating PRL levels. Analyses of PRLR expression in breast cancer have been limited by small sample sizes (<70 cases), no or partial risk factor information from the patients who contributed tissue to these studies, and use of varying methods for assessing PRLR, including *in situ* hybridization and reverse-transcriptase PCR for assessing mRNA expression and immunohistochemistry employing different reagent antibodies and staining protocols to assess protein levels [5, 14–19]. These analyses have generally not found significant relationships between PRLR protein expression and histological type, grade, size or node status [14, 15, 18, 19]. These reports also yielded mixed results regarding the association of PRLR expression with estrogen receptor expression [14, 15, 18].

With the emergence of elevated circulating PRL levels as an independent breast cancer risk factor, it is important to know whether PRLR expression is associated with specific tumor characteristics. Here we examine the associations of PRLR protein expression with tumor characteristics among cases in a large population-based case-control study conducted in Poland. Given that associations between PRLR status and other tumor characteristics could be modified by circulating PRL levels, we repeated these analyses for tumors cross-classified by PRL levels and PRLR expression when possible because this has not been well explored.

Methods

Study population

The population and design of the Polish case-control study has been reported elsewhere [20]. Participants provided written informed consent and the study protocol was approved by ethical review boards in Poland and the United States. Briefly, eligible cases were women ages 20–74 years diagnosed with pathologically confirmed breast cancer from 2000 to 2003 while living in Warsaw or Lodz, Poland. The Polish Electronic System, a database with demographic information of Polish citizens was used to randomly select controls, defined as women without breast cancer, frequency matched to cases on city and age in 5-year categories. In total, 2,386 cases (79% of eligible) and 2,502 controls (69% of eligible) consented to participate in an interview regarding breast cancer risk factors. Of the 2386 cases identified, 2143 were found to have an invasive diagnosis. Paraffin-embedded tumor tissue from 1437 (67% of invasive cases) was prepared as tissue microarrays (TMAs). Of these 1437 cases, 336 cases had missing cores leaving 1101 cases for PRLR analyses.

Pathology and immunohistochemical staining

Histopathological features, including histology, grade (based on modified Elston grading criteria that include architecture, nuclear atypia and mitotic rate), tumor size, and axillary lymph node metastases were assessed using surgical pathology reports and independent review (M.E.S.). Invasive breast cancers were prepared as TMAs with duplicate representation as 0.6-mm diameter cores (Beecher Instruments). The methods and results for immunohistochemical stains for estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (HER2) were reported previously [21–23]. In brief, IHC staining was done with antigen retrieval prior to antibody incubation according to established protocols for ER- α (clone 6F11, 1:200; Novocastra, Newcastle upon Tyne, United Kingdom), PR (clone PgR636, 1:1,000; Dako), and HER2 (polyclonal, 1:2,000; DakoCytomation, Glostrup, Denmark). The percentage (0–100%) and intensity (0 = negative, 1 = weak, 2 = intermediate, and 3 = strong) of tumor cells stained were recorded for each marker. ER and PR were classified as positive if the product of intensity and percentage was >10 . HER2 was considered positive if intermediate or strong staining was identified in at least 20% of tumor cells. In a prior study, ER, PR, and HER2 status also were assessed using automated quantitative analysis (AQUA) method [23]. In this prior publication, the AQUA and semi-quantitative immunohistochemistry methods were highly correlated for ER ($r_s^2=0.80$), PR ($r_s^2=0.86$), and HER2 ($r_s^2=0.87$). The AQUA data were utilized here to examine ER, PR, and HER2 status using a continuous measure and compare these results to those determined from microscopic assessment of semi-quantitative immunohistochemistry.

PRLR immunohistochemical (IHC) staining was performed using a mouse anti-PRLR extracellular domain (ECD) antibody (Cat# 35-9200; Invitrogen, Camarillo, CA). This antibody has been shown to be the most specific for the PRLR, out of a panel of six putative PRLR antibodies tested, and demonstrated potential immunoreactivity in human breast carcinomas when incubated with slides overnight at 4°C, at a concentration of 3 $\mu\text{g/ml}$ [24]. Here, the anti-PRLR ECD antibody was applied at 10 $\mu\text{g/ml}$ for 1 hr at RT, followed by detection using the anti-Mouse Vectastain Elite ABC kit protocol (Vector Laboratories, Burlingame, CA). This kit utilizes diaminobenzidine (DAB) as a substrate for the peroxidase enzyme. The resulting staining is a brown precipitate which permits categorizing the staining as negative, equivocal, weakly positive, and strongly positive. A pathologist (M.D.) scored all tumor samples using a digital imaging system. Scores were based on intensity only, as this was the predominant factor that differed across the categories. For tumors with multiple cores on the TMAs, scoring was based on simultaneous review of staining patterns

across all cores. Staining patterns were largely homogenous across the cores; therefore, scoring was assigned by the pathologist based on overall impression of the staining across the cores. Intra-rater agreement on 23 tumors with duplicate representation on the TMA was 74%. The category of “weakly positive” was the most inconsistent across the duplicate readings; therefore, PRLR staining was characterized as a dichotomous variable – strongly positive (referred to as “positive”) compared to weakly positive/equivocal/negative (referred to as “negative”) for subsequent analyses (Supplementary Data - Figure 1). A categorical variable was created that combined PRL levels and PRLR expression into four categories: low PRL/negative PRLR; low PRL/positive PRLR; high PRL/negative PRLR; and high PRL/positive PRLR.

Statistical analysis

Tumor tissue staining of PRLR could not be assessed as determined by a pathologist (MD) for 365 of the 1101 cases, due to inadequate tumor tissue representation or digital image quality. In total 735 invasive cases with PRLR data were available for analyses. χ^2 analyses were used to determine if tumor characteristics varied between those tumors for which there was PRLR reading and those for which a PRLR reading was not available. In comparing tumors with PRLR expression data to those without, the tumors not included in the analyses were more likely to be of lobular histology ($p=0.02$), less than 2cm ($p=0.03$) and HER2 negative ($p=0.03$). All other tumor characteristics reported here were not statistically different across the two groups.

Comparisons across strata of tumor characteristics by PRLR status were also assessed using χ^2 analyses. Tumor characteristics significantly associated with PRLR staining in univariate analyses were included in multivariate logistic regression analyses with the relevant tumor characteristics as the predictors and PRLR staining (negative vs. positive) as the outcome variable. Exploratory case-case comparisons examining associations between risk factors and PRLR staining were also conducted using χ^2 analyses. Statistical significance was defined at two-sided $p<0.05$ and all analyses were completed with SAS (version 9.0, SAS Institute, Inc., Cary, NC) or STATA (version 9.0) software.

Results

Characteristics of Population

The demographics of the 142 pre- and 594 post-menopausal women included in this analysis are reported in Table 1. Using the dichotomous variable for PRLR staining that was based on grouping the categories of negative, equivocal, and weak together as “low/negative” PRLR expression, 83% of tumors in this sample were scored as strong PRLR staining (Table 2). The proportion of tumors staining positive was similar across both pre- and post-menopausal cases; therefore, data for these groups were combined.

Relationships between Prolactin Receptor Status and Pathological Characteristics

PRLR negative status was significantly associated with larger tumor size ($p=0.0005$) and poorly differentiated histological grade ($p=1.2\times 10^{-08}$) (Table 2). A non-significant but suggested relationship was found between PRLR negative and HER2 positive status ($p=0.08$). Other pathological factors were unrelated to PRLR status both in the overall population (Table 2) and when stratified by menopausal status (Table 3). The associations of PRLR expression with size and differentiation were similar for tumors cross-classified by menopausal status and ER status, except for the small strata of ER negative premenopausal women, which was limited to 44 cases (Table 4).

Additional analyses of 579 women with data for size, grade, ER status and PRLR using multivariate logistic regression demonstrated that PRLR negative status was associated with larger tumors ($p=0.01$, Odds Ratio [OR]=1.81, 95% confidence interval [CI] 1.14, 2.86) and poor differentiation ($p=0<0.0001$, OR=3.13, 95% CI 1.94, 5.03), independent of ER status. In these models, PRLR staining was not associated with ER status ($p=0.51$, OR=1.18, 95% CI 0.74, 1.88). Stratified analyses of data for premenopausal women was limited by sample size ($n=109$).

In addition to data from standard microscopic assessment of semi-quantitative immunohistochemistry methods, almost all the 579 women also had data from the AQUA method for ER ($n=564$), PR ($n=561$) and HER2 ($n=557$) [23]. Including these continuous data in the regression models did not change the outcome of the association of PRLR negative status with larger tumors ($p=0<0.0001$, Odds Ratio [OR]=1.81, 95% confidence interval [CI] 1.14, 2.89) and poor differentiation ($p=0<0.0001$, OR=2.89, 95% CI 1.78, 4.63), independent of ER status.

Approximately 349 cases in this analysis were included in a prior report assessing associations with circulating PRL levels [8]. The current data do not show an association between PRL levels and PRLR expression (Table 2). The cross-classification of circulating PRL levels with PRLR staining did not reveal stronger associations than found for analyses of PRLR staining alone.

Association between Breast Cancer Risk Factors and Tumor PRLR Status among Cases

Increasing age at first birth was associated with positive PRLR staining ($p=0.03$) and increasing BMI was associated with negative PRLR status ($p=0.002$) (Table 5). These results were similar among postmenopausal women ($n=467$) but were weaker among premenopausal women ($n=109$) (data not shown). Other risk factors were unrelated. When BMI was added to the multivariate model containing tumor size, grade, and ER status, BMI remained independently associated with PRLR expression. Therefore, the associations of PRLR with tumor size, grade, and patient BMI are independent of each other and ER status.

Discussion

In this study, breast cancers negative for PRLR expression were larger and more poorly differentiated than breast cancers with positive PRLR expression, independent of ER status, other tumor characteristics and such patient characteristics as BMI. Similarly, previous studies have found that PRLR expression is independent of ER status (13–16). Here, ER status was reported using microscopic assisted semi-quantitative immunohistochemistry and a score of positive or negative determined from methods outlined in prior publications based on this population [21–23]. The results of the associations of PRLR negative staining with larger and more poorly differentiated tumors, independent of ER status, also were examined using the continuous AQUA data. Including the continuous measures of ER, PR or HER2 staining in the multivariate models here did not change the outcome.

The associations between high levels of PRLR, smaller tumor size and lower grade suggests that PRLR may be a marker of better differentiation and may be involved in this phenotype. These results are consistent with data demonstrating breast cancer xenografts grown in immunocompromised mice upregulated expression of genes involved in differentiation pathways when stimulated *in vivo* with prolactin [25]. Similarly, phosphorylation of a key signaling component of the prolactin receptor pathway, Stat5, is associated with more favorable prognosis with regard to breast cancer outcomes [26–30]. We are unable to examine the association of PRLR expression with breast cancer outcomes because of limited number of deaths in follow-up. Therefore, it remains unclear whether PRLR staining

provides additional prognostic information beyond that contributed by other factors. In addition, examining markers of PRLR activation, such as Stat5 activation, may permit further refinement of the strong PRLR staining category by separating tumors that highly express PRLR from tumors that both highly express PRLR and exhibit characteristics associated with PRLR activation.

A limitation of this study is that it was only possible to score PRLR dichotomously, which, in combination with the low percentage of negative cases, limited statistical power. Our estimate of PRLR negative cases was conservative whereby PRLR negative scores were restricted to cancers in which both TMA cores were scored negative. Prior publications by Camp and colleagues demonstrated that immunohistochemical staining scores assigned based on duplicate representation in a tissue microarray correlate with staining assigned from review of whole sections in >95% of breast cancer cases [31].

Another factor in this analysis was the use of a PRLR reagent antibody for immunohistochemistry that binds to the extracellular domain of the receptor and detects multiple forms of the PRLR. There are six different PRL receptors that arise from alternative transcripts of the *PRLR* gene, which have different functions. [5, 17, 32–35]. The most well characterized isoform is the long-form (i.e. 90 kDa) of the receptor, which initiates the Jak2/Stat5, PI3K, and MAPK signaling pathways, resulting in enhanced survival, proliferation and differentiation of breast epithelial cells [5]. Other isoforms of the PRLR may be negative inhibitors of signaling (16, 24).

The results presented here, in combination with those from prior studies demonstrating associations between circulating PRL (i.e. ligand) levels and selected breast cancer risk factors (5, 8–12), provide a basis for additional epidemiologic studies extending these results to other aspects of the PRL/PRLR signaling network. While several large studies have now examined circulating PRL levels, this report is the first to look at the association of circulating PRL levels with PRLR expression but could do so only in a subset of cases. These associations should be examined in larger, prospective studies. There also is the unresolved question of autocrine PRL production, including whether this could result in locally higher concentrations of the hormone and have implications for PRLR signaling [36].

Conclusions

The study reported here is the largest to evaluate the associations of PRLR expression with tumor characteristics and the findings of associations between negative PRLR staining with larger tumor size and poorly differentiated histological grade are interesting. However, replication and extension of these results to include other relevant markers, including expression and/or activation of specific prolactin receptor isoforms or downstream signaling molecules, and follow-up would be useful to understand whether PRLR expression has clinical importance beyond established factors such as tumor size and grade. Future studies using assays that enable more detailed characterization of the PRLR may increase our knowledge of the importance of this receptor in breast cancer.

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Fig 1. Tumor tissue microarray (TMA) images of prolactin receptor expression in human breast cancer

Immunohistochemical staining representing a.) negative/low PRLR tumor tissue expression and b.) strong positive PRLR tumor tissue expression.

Table 1

Characteristics of 736 cases by menopausal status from the Polish Breast Cancer Study

	Premenopausal Cases N=142		Postmenopausal Cases N=594	
	Mean	SD	Mean	SD
Age, years	44.9	5.7	61.2	7.9
Age at menarche, years	13.0	1.7	13.6	1.7
Age at first birth, years	24.3	4.8	24.1	4.6
Age at menopause, years	-----	-----	49.7	4.7
Parous, Frequency [†]	128	90.1	501	84.3
Family history of breast cancer, Frequency [†]	14	9.9	69	11.6
History of benign breast disease, Frequency [†]	35	24.7	105	17.7
HRT use, Frequency [†]	-----	-----	148	24.9

[†] Frequencies represented as N and % instead of Mean and SD

Association of tumor characteristics with prolactin receptor expression among 736 women with breast cancer (pre- and postmenopausal) from the Polish Breast Cancer Study

Table 2

	PRLR staining (negative or low) N (%)		PRLR staining (strong) N (%)	P Value*
Menopausal status N=736				
Premenopausal	29 (20.4)		113 (79.6)	
Postmenopausal	98 (16.5)		496 (83.5)	0.27
PRL serum levels** N=349				
Low	30 (17.9)		138 (82.1)	
High	30 (16.9)		148 (83.2)	0.81
Histological type N=713				
Ductal	106 (17.6)		488 (82.2)	
Lobular	17 (14.3)		102 (85.7)	0.35
Tumor size N=715				
2 cm	42 (11.9)		310 (88.1)	
>2 cm	79 (21.8)		284 (78.2)	0.0005
Tumor grade N=595				
Well/moderately differentiated	35 (10.8)		288 (89.2)	
Poorly differentiated	80 (29.4)		192 (70.6)	1.2×10 ⁻⁰⁸
Axillary node metastasis N=716				
Node negative	62 (15.7)		333 (84.3)	
Node positive	60 (18.7)		261 (81.3)	0.29
ER N=732				
Negative	50 (20.1)		199 (79.9)	
Positive	76 (15.7)		407 (84.3)	0.14
PR N= 731				
Negative	70 (18.4)		311 (81.6)	
Positive	56 (16.0)		294 (84.0)	0.40
HER2 N=721				
Negative	104 (16.3)		534 (83.7)	
Positive	20 (24.1)		63 (75.9)	0.08

	PRLR staining (negative or low) N (%)	PRLR staining (strong) N (%)	P Value*
ER/PR N=730			
ER+ PR+	49 (15.6)	265 (84.4)	
ER+ PR-	27 (16.1)	141 (83.9)	
ER- PR+	7 (19.4)	29 (80.6)	
ER- PR-	43 (20.3)	169 (79.7)	0.15
ER/PR/HER2 N=721			
ER+ or PR+ and HER2-	78 (15.5)	425 (84.5)	
ER+ or PR+ and HER2+	5 (35.7)	9 (64.3)	
ER- and PR- and HER2+	9 (17.3)	43 (82.7)	
ER- and PR- and HER2-	32 (21.1)	120 (78.9)	0.11

Not all strata sum to 100% due to rounding.

* p values from χ^2 test

** category for PRL levels assigned using median PRL levels for the subset of cases with this measurement

Table 3

Association of tumor characteristics with prolactin receptor expression among 736 women (stratified by menopausal status) with breast cancer from the Polish Breast Cancer Study

	Premenopausal N=142			Postmenopausal N=594		
	PRLR staining (negative or low) N (%)	P value	PRLR staining (strong) N (%)	PRLR staining (negative or low) N (%)	P value	P value *
PRL serum levels ** N=349						
Low	5 (17.9)		23 (82.1)	25 (17.9)		115 (82.1)
High	7 (23.3)	0.61	23 (76.7)	23 (15.5)		125 (84.5)
Histological type N=713						0.60
Ductal	27 (22.3)		94 (77.7)	79 (16.7)		394 (83.3)
Lobular	1 (7.1)	0.30	13 (92.7)	16 (15.2)		89 (84.8)
Axillary node metastasis N=716						0.71
Node negative	13 (19.7)		53 (80.3)	49 (14.9)		280 (85.1)
Node positive	15 (20.6)	0.90	58 (79.5)	45 (18.2)		203 (81.9)
ER N=732						0.30
Negative	12 (19.7)		49 (80.3)	38 (20.2)		150 (79.8)
Positive	17 (21.3)	0.82	63 (78.8)	59 (14.6)		344 (85.4)
PR N= 731						0.09
Negative	14 (22.2)		49 (77.8)	56 (17.6)		262 (82.4)
Positive	15 (19.2)	0.66	63 (80.8)	41 (15.1)		231 (84.9)
HER2 N=721						0.41
Negative	25 (20.5)		97 (79.5)	79 (15.3)		437 (84.7)
Positive	4 (22.2)	1.0	14 (77.8)	16 (24.6)		49 (75.4)
ER/PR N=730						0.06
ER+ PR+	13 (20.3)		51 (79.7)	36 (14.4)		214 (85.6)
ER+PR-	4 (25.0)		12 (75.0)	23 (15.1)		129 (84.9)
ER-PR+	2 (14.3)		12 (85.7)	5 (22.7)		17 (77.3)
ER-PR-	10 (21.3)	0.99	37 (78.7)	33 (20.0)		132 (80.0)
ER/PR/HER2 N=721						0.11
ER+ or PR+ and HER2-	18 (19.6)		74 (80.4)	60 (14.6)		351 (85.4)
ER+ or PR+ and HER2+	1 (50.0)		1 (50.0)	4 (33.3)		8 (66.7)

	Premenopausal N=142		Postmenopausal N=594		P value	P value*
	PRLR staining (negative or low) N (%)	PRLR staining (strong) N (%)	PRLR staining (negative or low) N (%)	PRLR staining (strong) N (%)		
ER- and PR- and HER2+	2 (16.7)	10 (83.3)	7 (17.5)	33 (82.5)		
ER- and PR- and HER2-	8 (24.2)	25 (75.8)	24 (20.2)	95 (79.8)	0.64	0.13

Not all strata sum to 100% due to rounding.

* p values from χ^2 test

** category for PRL levels assigned using median PRL levels for the subset of cases with this measurement

Table 4

Association of tumor size and grade with prolactin receptor expression stratified by ER status among 736 women with breast cancer from the Polish Breast Cancer Study

	Overall Population N=736			Premenopausal N=142			Postmenopausal N=594		
	PRLR staining (negative or low) N (%)	PRLR staining (strong) N (%)	P Value*	PRLR staining (negative or low) N (%)	PRLR staining (strong) N (%)	P Value*	PRLR staining (negative or low) N (%)	PRLR staining (strong) N (%)	P Value*
ER+ tumors									
Tumor size									
2 cm	31 (11.9)	230 (88.1)		7 (16.7)	35 (83.3)		24 (11.0)	195 (89.0)	
>2 cm	41 (19.5)	169 (80.5)	0.02	9 (25.7)	26 (74.3)	0.33	32 (18.3)	143 (81.7)	0.04
Tumor grade									
Well/moderately differentiated	26 (10.2)	230 (89.8)		5 (12.2)	36 (87.8)		21 (9.8)	194 (90.2)	
Poorly differentiated	45 (31.7)	97 (68.3)	2.2×10 ⁻⁰⁷	11 (40.7)	16 (59.3)	0.007	34 (29.6)	81 (70.4)	9.3×10 ⁻⁰⁶
ER- tumors									
Tumor size									
2 cm	10 (11.4)	78 (88.6)		2 (8.0)	23 (92.0)		8 (12.7)	55 (87.3)	
>2 cm	38 (25.0)	114 (75.0)	0.01	10 (29.4)	24 (70.6)	0.04	28 (23.7)	90 (76.3)	0.08
Tumor grade									
Well/moderately differentiated	8 (12.3)	57 (87.7)		4 (25.0)	12 (75.0)		4 (8.2)	45 (91.8)	
Poorly differentiated	35 (26.9)	95 (73.1)	0.02	7 (25.0)	21 (75.0)	1.0	28 (27.5)	74 (72.6)	0.007

* p values from χ^2 test

Table 5

Association of risk factors with prolactin receptor expression among 736 women with breast cancer (pre- and postmenopausal) from the Polish Breast Cancer Study

	PRLR staining (negative or low) N (%)		PRLR staining (strong) N (%)		P Value**
Parity N=736					
Nulliparous	19 (17.8)		88 (82.8)		
Parous	108 (17.2)		521 (82.8)		0.88
Age at first birth* N=628					
<20 years	16 (21.3)		59 (78.7)		
20-24	63 (19.7)		257 (80.3)		
25-30	19 (12.5)		133 (87.5)		
30+	10 (12.4)		71 (87.7)		0.03
Number of full term births* N=629					
1	42 (16.2)		217 (83.8)		
2	52 (18.0)		237 (82.0)		
3+	14 (17.3)		67 (82.7)		0.69
Age at last birth* N=629					
<24 years	30 (20.3)		118 (79.7)		
24-27	25 (18.4)		111 (81.6)		
27-31	26 (16.2)		135 (83.9)		
31+	27 (14.7)		157 (85.3)		0.15
Years since last birth* N=629					
<22.5 years	30 (19.0)		128 (81.0)		
22.5-31.0	28 (19.3)		117 (80.7)		
31.0-40.0	22 (14.0)		135 (86.0)		
40.0+	28 (16.6)		141 (83.4)		0.36
Number of months breastfeeding* N=629					
Never	30 (22.1)		106 (77.9)		
< 6 months	46 (13.8)		287 (86.2)		
6-11	17 (18.1)		77 (81.9)		
12-23	15 (22.7)		51 (77.3)		0.85

	PRLR staining (negative or low) N (%)	PRLR staining (strong) N (%)	P Value**
BMI category N=733			
<25	29 (12.4)	205 (87.6)	
25-<30	34 (13.4)	220 (86.6)	
30+	62 (25.3)	183 (74.7)	0.002

Not all strata sum to 100% due to rounding.

* analyses conducted among only parous women

** p values from χ^2 test