# PRR11 expression in early ER+/HER2-low breast cancer: Association with estrogen receptor positivity and exploratory analysis of prognostic significance

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**Abstract.** The present study aimed to investigate proline-rich protein 11 (PRR11) expression and to exploratorily assess its prognostic relevance in early estrogen receptor (ER)+/HER2-low breast cancer. Data of 124 patients with early ER+/HER2-low breast cancer were evaluated retrospectively. PRR11 expression was analyzed in tumor tissues and reported as the median fold change relative to the cohort median. PRR11 expression was comparatively analyzed in subgroups according to ER percentages in 10% intervals and HER2-low status: CerbB2 immunohistochemistry (IHC) +1 (n=66) and +2 (n=58). PRR11 expression analysis was also performed in the residual tumors of patients who received neoadjuvant chemotherapy (NAC; n=14) and patients without pathological complete response (pCR; n=11). The median PRR11 expression fold change was 0.31 in the ER (1-10%) subgroup, 0.50 in the ER (20-30%) subgroup, 1.19 in the ER (40-50%) subgroup, 1.23 in the ER (70-80%) subgroup, 1.41 in the ER (80-90%) subgroup and 1.55 in the ER (>90%) subgroup. The median fold change in PRR11 expression was 1.717 in the CerbB2 IHC 1+ subgroup and 0.999 in the CerbB2 IHC 2+ subgroup. PRR11 expression was decreased in 4 patients (36%) and increased in 7 patients (64%) after NAC. Kaplan-Meier survival analysis stratified by PRR11 expression did not indicate a statistically significant difference in 5-year disease-free survival (DFS) or overall survival between the high- and low-PRR11 expression groups. Receiver operating characteristic analysis of DFS yielded an area under the curve of 0.409 (optimal cut-off, 0.18; sensitivity, 100%; specificity, 15.3%), indicating no meaningful discriminatory value. There was a positive association between PRR11 expression and the ER positivity rate. Higher PRR11 levels in residual tumors of non-pCR patients represented a preliminary, hypothesis-generating observation regarding the potential predictive value of PRR11 in patients receiving NAC. Randomized clinical trials are needed in this area.

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## Introduction

The proline-rich protein 11 (PRR11) gene is located on human chromosome 17q22-23 and comprises 10 exons and nine introns (1,2). PRR11 may serve a role in carcinogenesis across various cancer types (3-6). PRR11 may contribute to prognosis and treatment outcomes in solid tumors, such as breast cancer, colon cancer, gastric cancer, hepatocellular carcinoma, cholangiocellular carcinoma, pancreatic cancer, lung cancer, ovarian cancer and osteosarcoma (7). In numerous malignancies, cell cycle defects are considered to be key to carcinogenesis. Ji *et al* (2) reported that PRR11 expression increases from the $G_1$ phase to the $G_2$/M phase of the cell cycle. PRR11 overexpression may contribute to cell proliferation. Marked inhibition of cell proliferation was also detected after PRR11 depletion. This outcome suggests that PRR11 may serve a role in the cell cycle, particularly in S-phase progression and subsequent cell cycle steps (2).

Breast cancer remains the most commonly diagnosed cancer among women worldwide, with ~2.3 million women diagnosed and 670,000 mortalities globally in 2022 (ref). Breast cancer is a biologically heterogeneous disease, and therapeutic strategies are increasingly tailored to distinct molecular subtypes, ranging from immunotherapeutic approaches under investigation in triple-negative disease to HER2-directed antibody-drug conjugates in HER2-low disease (8). Luminal-like breast cancer is defined as HER2-negative or HER2-positive hormone receptor-positive breast cancer. Previously, HER2-negative disease has been defined as disease with a CerbB2 immunohistochemistry (IHC) score of 0 or an IHC

score of 1+ or 2+ without HER2 amplification based on *in situ* hybridization (ISH) (7). However, it is well-established that IHC score 1 or score 2+ and no HER2 amplification based on ISH are distinct entities and are referred to as HER2-low disease (7). HER2-low status is prognostic and predictive for treatment response for novel therapeutics, such as trastuzumab deruxtecan, an antibody-drug conjugate targeting HER2 (9). However, there are numerous gray areas in understanding and addressing treatment sensitivity and/or resistance in these patients. The prognostic value of PRR11 expression has not been well-documented in patients with estrogen receptor (ER)$^+$/HER2-low breast cancer, representing an important gap in the existing literature that the present study aims to address. ER$^{+/}$HER2-low breast cancer represents a large and clinically important luminal subgroup whose therapeutic landscape has expanded with the introduction of HER2-directed antibody-drug conjugates (9). Despite this, the molecular determinants of endocrine response and resistance within this specific subtype remain incompletely characterized. Given its established mechanistic link to PI3K signaling and antiestrogen resistance in ER-positive disease, PRR11 represents a biologically plausible candidate marker in this setting, providing the rationale for the present exploratory investigation.

The present study aimed to investigate PRR11 expression and its effects on prognosis in patients with localized or locally advanced-stage (non-metastatic), ER$^+$ and HER2-low breast cancer.

## Materials and methods

*Study design*. A total of 124 patients with non-metastatic ER$^+$/HER2-low breast cancer followed up at Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital (Ankara, Turkey) between January 2010 and December 2019 were analyzed retrospectively in February 2024. ER-positive (ER, ≥1%) and HER2-low patients were included. The cut-off level for ER positivity was 1%. For the primary grouping analysis, patients were categorized into three ER subgroups: ER, 1-50%; ER, 51-89%; and ER, ≥90%. For the detailed PRR11 expression analysis, patients were additionally analyzed in 10% ER intervals to examine the gradient relationship between the ER positivity rate and PRR11 expression levels. The 10% ER interval analysis was adopted to enable a fine-grained, gradient assessment of the relationship between ER positivity and PRR11 expression. ER expression is reported as a continuous percentage (1-100%) in routine pathology, and accumulating evidence indicates that ER positivity behaves as a biological continuum rather than a dichotomous variable, with low-ER (1-10%) tumors being molecularly and clinically distinct from high-ER tumors (10,11). Decile-based stratification therefore permits the detection of dose-response patterns that coarser categorical grouping may obscure. HER2-low status was defined as a CerbB2 IHC score of 1+ or a CerbB2 IHC score of 2+ without HER2 amplification based on silver ISH (SISH). For analysis, HER2-low patients were further divided into the CerbB2 IHC score 1+ subgroup and the CerbB2 IHC score 2+ and SISH-negative subgroup.

Demographic characteristics of the patients such as age, sex, menopausal status and comorbidities, pathological characteristics such as pathological subtype, grade, Ki-67 value, ER percentage, progesterone receptor (PR) percentage and CerbB2 IHC score, and clinical characteristics such as the treatment modalities and survival outcomes for disease-free survival (DFS) and overall survival (OS) were recorded. DFS was defined as the interval between the diagnosis date and the date of recurrence or mortality. OS was defined as the interval between diagnosis and mortality or the last known date of being alive.

*IHC*. ER and HER2 expression was evaluated by IHC using formalin-fixed, paraffin-embedded (FFPE) tissue sections. All specimens were fixed for 24-72 h in room temperature in neutral buffered 10% formalin. Sections at 4 $\mu$m thick were prepared on positive charged slides. Immunohistochemical staining was performed on an automated BenchMark ULTRA IHC/ISH platform. After conditioning in 95˚C for 8 min with Cell Conditioner #1, prediluted PATHWAY anti-HER2/neu (4B5) Rabbit Monoclonal Primary Antibody (cat. no. 05278368001; Roche Diagnostics) was incubated in 36˚C for 36 min. A secondary UltraView Universal DAB Detection Kit (cat. no. 05269806001; Roche Diagnostics) was applied. Hematoxylin counterstain was applied at 36˚C for 16 min and Bluing reagent at 36˚C for 4 min. Stained slides were imaged using a Nikon Optiphot 2 microscope. ER positivity was defined as nuclear staining in ≥1% of tumor cells, scored as a percentage (1-100%). HER2 expression was scored according to the 2018 American Society of Clinical Oncology/College of American Pathologists guidelines (7): IHC score 0, no staining or incomplete faint membrane staining in ≤10% of tumor cells; IHC score 1+, incomplete faint/barely perceptible membrane staining in >10% of tumor cells; IHC score 2+, weak to moderate complete membrane staining in >10% of tumor cells; or IHC score 3+, strong complete membrane staining in >10% of tumor cells. HER2-low status was defined as an IHC score of 1+ or an IHC score of 2+ with negative SISH.

*RNA extraction, cDNA synthesis and reverse transcription-quantitative PCR (RT-qPCR)*. Total RNA was obtained from FFPE tumor tissue blocks using the DiaRex® Total RNA Extraction kit (cat. no. TR-0877; Diagen İnsan Sağlığı). Briefly, after 5-30 mg of tissue was homogenized in a 1.5-ml homogenization tube, extraction was performed according to the kit manufacturer's instructions, and finally, 30-50 $\mu$l total RNA was obtained. Total RNA was stored at -80˚C until analysis.

Preserved samples were thawed on ice, and RNA concentrations were measured spectrophotometrically (Colibri; Berthold Technologies GmbH & Co.) to standardize RNA input prior to cDNA synthesis. cDNA synthesis was performed using the SOLIScript® RT cDNA Synthesis Kit (Solis BioDyne) according to the manufacturer's protocol. RT was performed at 50˚C for 5 min, followed by enzyme inactivation at 85˚C for 5 min. cDNA products were stored at -20˚C until use.

PRR11 mRNA expression levels were quantified using the $2^{-\Delta\Delta Cq}$ method (12), with β-actin (ACTB) serving as the endogenous reference gene for normalization. β-actin (ACTB) was selected as the endogenous reference gene because it is a constitutively expressed housekeeping gene that is widely used and validated for the normalization of qPCR data in breast

tumor tissue, including FFPE material, and displays acceptable expression stability across breast cancer specimens, including estrogen receptor-positive tumors (13). The present study acknowledges that the use of a single reference gene, rather than a panel of validated controls, represents a methodological limitation. SolisFAST® SolisGreen® qPCR Mix (no ROX), 5X (Solis BioDyne) was used. Each 20 $\mu$l reaction consisted of 4 $\mu$l master mix, 5 $\mu$l primer mix (containing 0.3 mM forward and reverse primers), 6 $\mu$l distilled water and 5 $\mu$l cDNA. Amplification was performed using a RT-qPCR system (BioRAD CFX-96; Bio-Rad Laboratories, Inc.) with the following protocol: Initial denaturation at 95˚C for 5 min, followed by 45 cycles of 95˚C for 5 sec and 57˚C for 30 sec. The primer sequences were as follows: PRR11 forward, 5'-GAGTCGGTATTTCTTCAAT-3' and reverse, 5'-CTAAACTCTGGGTTATGC-3'; and ACTB forward, 5'-TGAAGATCAAGATCATTGCT-3' and reverse, 5'-ATACTCCTGCTTGCTGAT-3'. Expression values were reported as the fold change relative to the cohort median (median fold change) and patients were categorized as having high or low PRR11 expression based on this median threshold. Relative mRNA expression levels were calculated using the $2^{-\Delta\Delta Cq}$ method using an R package (version 4.3.1; R Foundation for Statistical Computing; qpcrtools 1.0.1; ggpubr 0.6.0; dplyr 1.1.4; tidyverse 2.0.0; car 3.1-2).

*Statistical analysis*. Descriptive statistics were used to examine the demographic and clinicopathological characteristics of the patients. Categorical variables were compared using the $\chi^2$ test; when >20% of cells had expected counts below 5, Fisher's exact test (Freeman-Halton extension) was used instead. The unpaired Student's t-test was used to compare two groups when the data were normally distributed.

The median follow-up duration was calculated using the reverse Kaplan-Meier method. Survival analyses of 5-year DFS and 5-year OS were performed using the Kaplan-Meier method and possible prognostic factors were compared by log-rank test. Patients were stratified into high PRR11 expression (above median fold change) and low PRR11 expression (at or below median fold change) groups for survival analyses. Receiver operating characteristic (ROC) analysis was performed to evaluate the discriminatory ability of PRR11 expression for DFS and OS outcomes, with optimal cut-off values determined using the Youden index. DFS was defined as the time from the date of diagnosis to the date of recurrence, if any, or the date of last follow-up. OS was defined as the time from the date of diagnosis to the date of exitus or last follow-up. All statistical analyses were performed using SPSS version 21.0 (IBM Corp.). P<0.05 was considered to indicate a statistically significant difference.

## Results

*Baseline characteristics of the patients*. The mean age was 53.4 (±10.5) years. All patients were female. Of the patients, 51 (41.1%) were premenopausal and 73 (58.9%) were postmenopausal. In terms of pathological features, all patients had ER+/HER2-low breast cancer. A total of 14 patients (11.3%) had grade 1 breast cancer, 60 patients (48.4%) had grade 2 breast cancer and 46 patients (37.1%) had grade 3 breast cancer. The mean Ki-67 value was 29.3% (±17.4%). A total of 66 patients (53.2%) had a CerbB2 IHC score of 1+, while 58 patients (46.8%) had a score of 2+. The clinical and pathological characteristics are presented in Table I.

Table I. Baseline characteristics of patients.

| Characteristic | No. of individuals (n=124) |
|---|---|
| Mean age, years (±SD) | 53.4 (±10.5) |
| Sex, n | |
| Female | 124 |
| Male | 0 |
| Menopausal status, n (%) | |
| Premenopausal | 51 (41.1) |
| Postmenopausal | 73 (58.9) |
| Hypertension, n (%) | 36 (29) |
| Diabetes mellitus, n (%) | 22 (17.7) |
| Heart disease, n (%) | 6 (4.8) |
| Lung disease, n (%) | 3 (2.4) |
| Pathological subtype, n (%) | |
| IDC | 69 (55.6) |
| ILC | 7 (5.6) |
| Others | 48 (38.7) |
| Grade, n (%) | |
| 1 | 14 (11.3) |
| 2 | 60 (48.4) |
| 3 | 46 (37.1) |
| Mean Ki67, % (±SD) | 29.3 (±17.4) |
| Hormone receptors, % (±SD) | |
| ER | 87 (±21.1) |
| PR | 50.8 (±33.4) |
| CerbB2 score (IHC) | |
| Score 1 (1+) | 66 (53.2) |
| Score 2 (2+, SISH negative) | 58 (46.8) |
| Stage, n (%) | |
| 1A | 26 (21) |
| 1B | 1 (0.8) |
| 2A | 37 (29.8) |
| 2B | 37 (29.8) |
| 3A | 12 (9.7) |
| 3B | 1 (0.8) |
| 3C | 10 (8.1) |

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry.

The patients were divided into groups based on their CerbB2 scores. Demographic characteristics, including age, sex and comorbidities, and pathological features, including histopathological subtype, grade, Ki-67 value, ER percentage, PR percentage and stage, were compared between the groups of patients based on CerbB2 scores. These two groups had similar demographic and clinicopathological characteristics (Table II).

Table II. Baseline patients' characteristics according to CerbB2 IHC score.

| Characteristic | CerbB2 score 1 Total (n=66) | CerbB2 score 2 Total (n=58) | P-value |
|---|---|---|---|
| Age, years (±SD) | 53.4 (±10.5) | 53.4 (±10.6) | 0.148 |
| Sex F/M (n) | | | Not applicable |
| Female | 66 | 58 | |
| Male | 0 | 0 | |
| Menopausal status, n (%) | | | 0.250 |
| Premenopausal | 24 (36.4) | 27 (46.6%) | |
| Postmenopausal | 42 (63.6) | 31 (53.4%) | |
| Hypertension, n (%) | 19 (28.8) | 17 (29.3%) | 0.949 |
| Diabetes mellitus, n (%) | 11 (16.7) | 11 (19%) | 0.738 |
| Heart disease, n (%) | 5 (7.6 ) | 1 (1.7%) | 0.130 |
| Lung disease, n (%) | 2 (3%) | 1 (1.7%) | 0.637 |
| Pathological subtype, n (%) | | | 0.957 |
| IDC | 36 (54.5%) | 33 (56.9%) | |
| ILC | 4 (6.1%) | 3 (5.2%) | |
| Others | 26 (39.4%) | 22 (37.9%) | |
| Grade, n (%) | | | 0.304 |
| 1 | 7 (10.6%) | 7 (12.1%) | |
| 2 | 29 (43.9%) | 31 (53.4%) | |
| 3 | 29 (43.9%) | 17 (29.3%) | |
| Ki67, % (±SD) | 30.9 (±19.7) | 27.5 (14.3%) | 0.247 |
| Hormone receptors, % (±SD) | | | |
| ER | 85.3 (±22.9) | 88.9 (±19) | 0.595 |
| PR | 53.3 (±31.7) | 48 (±35.2) | 0.569 |
| Stage, n (%) | | | 0.716 |
| 1A | 14 (21.2%) | 12 (20.7%) | |
| 1B | 0 | 1 (1.7%) | |
| 2A | 21 (31.8%) | 16 (27.6%) | |
| 2B | 17 (25.8%) | 20 (34.5%) | |
| 3A | 6 (9.1%) | 6 (10.3%) | |
| 3B | 1 (1.5%) | 0 | |
| 3C | 7 (10.6%) | 3 (5.2%) | |

IDC, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; PR, progesterone receptor; IHC, immunohistochemistry.

The patients were also divided into three subgroups based on the ER percentage: ER, 1-50% (n=11); ER, 51-89% (n=47) and ER ≥90% (n=66). The relationship between ER distributions and CerbB2 scores was examined. A total of 8 patients (12.1%) with a CerbB2 IHC score of 1+ were in the ER 1-50% group, 24 (36.4%) were in the ER 51-89% group and 34 (51.5%) were in the ER >90% group. Among the patients with a CerbB2 IHC score of 2+ (SISH-negative), 3 (5.2%) patients were in the ER 1-50% group, 23 (39.7%) in the ER 51-89% group and 32 (55.2%) in the ER >90% group. There was no difference between the patients with a CerbB2 IHC score of 1+ and 2+ in terms of ER groups (P=0.404).

A total of 96 (77.4%) patients received adjuvant chemotherapy, 14 patients (11.3%) received neoadjuvant chemotherapy (NAC) and 14 patients (11.3%) received adjuvant endocrine treatment only (no chemotherapy). The pathological complete response (pCR) rate was 21.4% in 3 of 14 patients who received neoadjuvant treatment. According to the residual cancer burden (RCB) index (14), the neoadjuvant treatment response in 6 patients was classified as partial response (RCB-II). A total of 5 patients were resistant to neoadjuvant treatment (RCB-III). While 87 patients (70.2%) received adjuvant radiotherapy, 37 patients (29.8%) did not. All patients received adjuvant endocrine therapy, including tamoxifen or an aromatase inhibitor, plus a luteinizing hormone-releasing hormone analog, based on their menopausal status and comorbidities.

*PRR11 expression analysis.* PRR11 expression status was analyzed in relation to the percentage of ER and CerbB2 IHC scores. When evaluating PRR11 expression by ER percentage, patients were grouped into 10% ER intervals. The median PRR11 fold change in each subgroup was as follows: 0.31 in the ER 1-10% subgroup, 0.50 in the ER 20-30% subgroup, 1.19 in the ER 40-50% subgroup, 1.23 in the ER 70-80% subgroup,

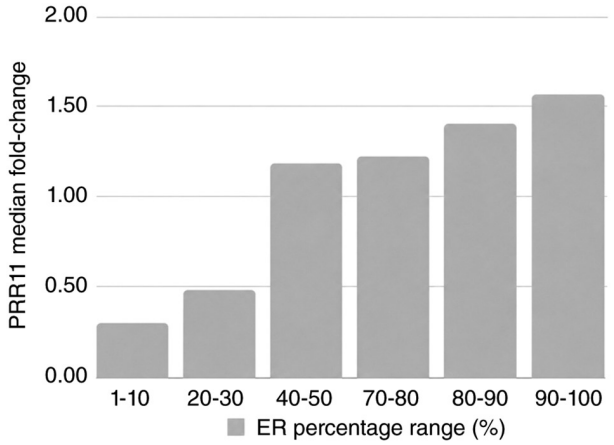


Figure 1. PRR11 expression levels according to ER intervals. ER, estrogen receptor.

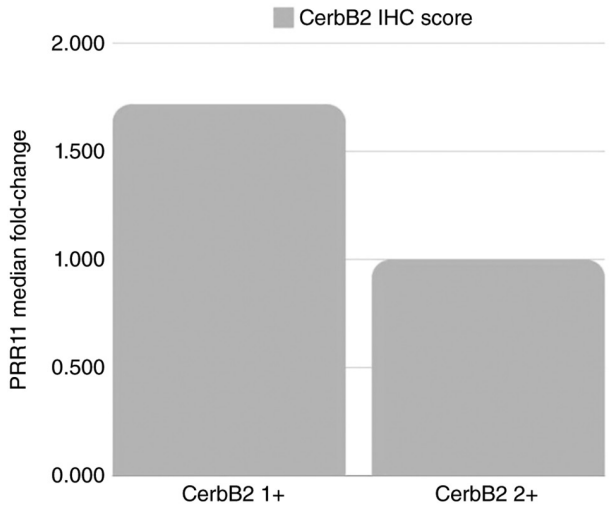


Figure 2. PRR11 expression levels according to CerbB2 IHC score. IHC, immunohistochemical.

1.41 in the ER 80-90% subgroup and 1.55 in the ER >90% subgroup (Fig. 1). A direct proportional relationship was observed between the ER positivity rate and PRR11 expression, particularly when the ER level was >40%.

PRR11 expression was also evaluated in two subgroups based on CerbB2 IHC scores: IHC score 1+ and IHC score 2+ (ISH-negative). While the median fold change for PRR11 was 1.717 in the CerbB2 IHC score 1+ subgroup, it was 0.999 in the CerbB2 IHC score 2+ subgroup (P=0.39; Fig. 2).

PRR11 expression was also evaluated in the tissues at diagnosis and in the postoperative residual tumors of 11 patients who did not achieve pCR with neoadjuvant treatment. After neoadjuvant treatment, PRR11 expression was decreased in 4 patients (36%) and increased in 7 patients (64%; Table III). However, exploratory paired analysis showed no statistically significant difference in median PRR11 fold change before and after neoadjuvant treatment (median, 2.318 vs. 1.540; Wilcoxon signed-rank test, P=0.465).

*Survival analysis*. The 5-year DFS and 5-year OS of the patients were evaluated. Survival analyses were performed

Table III. PRR11 expression before and after neoadjuvant treatment.

| | Median PRR11 fold change | |
|---|---|---|
| Patient no. | Before neoadjuvant treatment | After neoadjuvant treatment |
| 1 | 0.248 | 0,077 |
| 2 | 0.112 | 0.162 |
| 3 | 2.318 | 1.417 |
| 4 | 0.234 | 0.560 |
| 5 | 0.295 | 1.540 |
| 6 | 0.438 | 1.067 |
| 7 | 6.161 | 5.553 |
| 8 | 4.136 | 6.956 |
| 9 | 5.630 | 6.719 |
| 10 | 2.645 | 2.965 |
| 11 | 5.457 | 2.845 |

to examine potential prognostic factors affecting survival outcomes, including age, menopausal status, pathological subtype, grade, Ki-67 value, ER percentage range, CerbB2 IHC score and stage at diagnosis. None of these factors significantly affected 5-year DFS and 5-year OS in the univariate analysis (Table IV).

The 5-year DFS rate, evaluated in groups, based on ER percentage ranges, was 70% in the ER 1-50% subgroup, 93% in the ER 51-89% subgroup and 94.6% in the ER >90% subgroup (P=0.213). There was no significant difference in 5-year DFS between groups based on the CerbB2 IHC score (92.3% for the CerbB2 IHC score 1+ subgroup vs. 91% for the CerbB2 IHC score 2+ subgroup; P=0.317) (Table IV).

Survival analyses were also performed to compare patient outcomes according to PRR11 expression levels. Exploratory ROC analysis showed that PRR11 expression had no meaningful discriminatory ability for DFS (AUC=0.409). ROC analysis for OS was not considered reliable because of the limited number of events in this cohort (n=2), precluding robust curve estimation (data not shown). Patients were stratified into two groups based on the median PRR11 fold change: High PRR11 expression (above the median) and low PRR11 expression (at or below the median). Kaplan-Meier analysis revealed no statistically significant difference between the two groups in terms of 5-year DFS (P=0.272; data not shown). Formal log-rank testing for OS was not performed due to the insufficient number of events (n=2). These results indicated that PRR11 expression, as assessed in the present cohort, was not independently associated with survival outcomes in early-stage $ER^+$/HER2-low breast cancer.

## Discussion

PRR11 is expressed at low levels in normal tissues (15). PRR11 expression may serve a role in the cell cycle, particularly during the $G_2$/M phase, as well as in cell filopodia formation and motility (2,16). Therefore, PRR11 expression is being

Table IV. Univariate analysis of possible prognostic factors for survival.

| Characteristic | 5-year DFS, % | Median DFS (95% CI) | P-value | 5-year OS, % | Median OS (95% CI) | P-value |
|---|---|---|---|---|---|---|
| Age, years | | | 0.879 | | | 0.091 |
| <55 | 91.1 | NR | | 100 | NR | |
| ≥55 | 92.5 | NR | | 97.7 | NR | |
| Menopausal status | | | 0.515 | | | 0.171 |
| Premenopausal | 89 | NR | | 100 | NR | |
| Postmenopausal | 93.8 | NR | | 98.1 | NR | |
| Ki67, % | | | 0.240 | | | 0.809 |
| <20 | 87.3 | NR | | 97.3 | NR | |
| ≥20 | 96 | NR | | 100 | NR | |
| CerbB2 IHC score | | | 0.317 | | | 0.955 |
| +1 | 92.3 | NR | | 98 | NR | |
| +2 | 91 | NR | | 100 | NR | |
| Grade | | | 0.912 | | | 0.291 |
| Grade 1 and 2 | 92.3 | NR | | 98.3 | NR | |
| Grade 3 | 90 | NR | | 100 | NR | |
| ER range, % | | | 0.213 | | | 0.275 |
| 1-50 | 70 | NR | | 100 | NR | |
| 51-89 | 93 | NR | | 100 | NR | |
| ≥90 | 94.6 | NR | | 97.8 | NR | |
| Stage | | | 0.162 | | | 0.734 |
| 1 | 100 | NR | | 100 | NR | |
| 2A | 88.1 | NR | | 96.3 | NR | |
| 2B | 94.4 | NR | | 100 | NR | |
| 3 | 81.4 | NR | | 100 | NR | |
| Histopathological subtype | | | 0.934 | | | 0.608 |
| IDC | 92.7 | NR | | 98.1 | NR | |
| ILC | 85.7 | NR | | 100 | NR | |

OS, overall survival; DFS, disease-free survival; IHC, immunohistochemistry; ILD, invasive ductal carcinoma; ILC, invasive lobular carcinoma; ER, estrogen receptor; NR, not reached.

investigated as a potential prognostic marker and treatment target across numerous cancer types. Zhang *et al* (17) evaluated PRR11 expression levels in 33 different tumor types using The Cancer Genome Atlas (TCGA). The authors reported that PRR11 expression was upregulated in 19 cancer types, including breast cancer, and that PRR11 upregulation might be a poor prognostic factor in 10 cancer types but not in breast cancer. Wang *et al* (5) also analyzed RNA-sequencing data from TCGA to evaluate the prognostic significance of PRR11-spindle and kinetochore associated complex subunit 2 (SKA2) gene pair expression and p53 expression in breast cancer. The authors determined that the expression of the PRR11-SKA2 gene pair was lower in patients with breast cancer without p53 mutation when compared with those with p53 mutation, leading to improved DFS (P<0.0001) (5).

Breast cancer is a heterogeneous histopathological process with various pathological and molecular features. The prognostic value of PRR11 expression is not well-documented in patients with ER+/HER2-low breast cancer. In the present study, PRR11 expression levels were analyzed according to ER levels and CerbB2 IHC scores in patients with ER+/HER2-low early-stage (non-metastatic) breast cancer. A positive association was observed between PRR11 expression and the ER percentage. Notably, patients with higher ER percentages had higher PRR11 expression, since higher ER levels are a favorable prognostic factor in luminal-like breast cancer, while higher PRR11 expression is a poor prognostic factor based on the literature (5,18). Therefore, the coincidence of higher PRR11 expression and higher ER percentages presents a dilemma and requires further investigation.

One potential explanation for this positive association is that PRR11 may function, at least in part, as an ER-regulated gene. If PRR11 transcription is driven by ER signaling, its elevated expression in highly ER-positive tumors may not necessarily reflect aggressive tumor biology in the same manner as observed in ER-negative malignancies such as lung or gastric cancer. Although a formal analysis of ER-binding motifs within the PRR11 promoter region was beyond the scope of the present study, this hypothesis is biologically plausible given the known role of ER as a transcriptional activator (19).

A study by Lee *et al* (1) demonstrated that PRR11 overexpression amplified PI3K signaling and promoted antiestrogen resistance in ER-positive breast cancer, suggesting a bidirectional relationship between ER activity and PRR11 expression. Future studies incorporating chromatin immunoprecipitation or promoter analysis are warranted to determine whether PRR11 is a direct transcriptional target of ER signaling.

HER2-low breast cancer is a novel entity with prognostic and predictive value for anti-HER2 antibody-drug conjugates, such as trastuzumab deruxtecan. There are increasing data regarding differences in clinicopathological features and prognosis between patients with HER2-low and HER2-negative (CerbB2 score 0) breast cancer (20-22). To the best of our knowledge, there are no clear data on the relationship between PRR11 expression and HER2 status in breast cancer. In the present study, patients with a CerbB2 IHC score of 1+ had higher PRR11 expression when compared with patients with a CerbB2 IHC score of 2+, although this difference did not reach statistical significance (P=0.39). This trend, if significant in larger cohorts, could suggest that HER2-low breast cancer is not a biologically uniform entity and that IHC-based subcategorization may carry molecular relevance. The sample size limitation for this specific comparison (n=66 vs. n=58) is acknowledged, and larger cohorts are needed to determine whether this trend is reproducible. The limitation of relying solely on IHC/SISH for HER2-low classification, without correlation with HER2 mRNA levels, is also acknowledged as a direction for future research.

PRR11 expression levels did not exhibit a statistically significant association with 5-year DFS or OS in the present cohort. This finding contrasts with the reported prognostic significance of PRR11 in other malignancies, including lung, gastric and pancreatic cancer (4,6,23,24). Several factors may account for this discrepancy. First, the uniform administration of adjuvant endocrine therapy across the entire cohort may have masked the independent prognostic contribution of PRR11. Second, the relatively limited follow-up duration and the low event rate inherent to an early-stage patient population may have reduced the statistical power to detect a survival difference. Third, as aforementioned, PRR11 expression in highly ER-positive tumors may reflect ER-driven transcriptional activity rather than intrinsic oncogenic aggressiveness, attenuating its prognostic impact in this specific subtype. Furthermore, the exceptionally low mortality rate observed in this cohort (only 2 mortalities among 124 patients) reflects the favorable OS characteristic of early-stage ER$^+$/HER2-low breast cancer treated with standard multimodal therapy (25,26). While this represents a clinically favorable outcome, it substantially limits the statistical power to detect any survival difference attributable to PRR11 expression. It is therefore plausible that the OS of this patient population may have masked a potential prognostic effect of PRR11, should one exist. Larger cohorts with longer follow-up and higher event rates will be necessary to address this question adequately. A further limitation concerns the statistical modeling. Owing to the low number of events in this early-stage cohort (13 DSF events and 2 mortalities among 124 patients), a multivariable Cox proportional-hazards model could not be reliably constructed, as the resulting events-per-variable ratio would fall well below accepted thresholds for stable estimation. Consequently, the independent prognostic contribution of PRR11, adjusted for established clinicopathological covariates, could not be determined, and the present survival findings should be interpreted as univariate and exploratory.

PRR11 expression may also carry potential predictive value for treatment outcomes. Lee *et al* (1) reported an association between PRR11 overexpression and antiestrogen resistance via the PI3K signaling pathway in ER-positive breast cancer. In the present study, PRR11 expression was evaluated in residual tumor tissues from 11 patients who did not achieve a pCR following NAC. Post-treatment PRR11 expression levels were higher than pre-treatment levels in 7 of these 11 patients (64%). While this observation is intriguing and raises the hypothesis that elevated PRR11 expression in residual disease may reflect chemotherapy-resistant tumor clones or an adaptation mechanism contributing to treatment resistance, these findings must be interpreted with considerable caution. The sample size of 11 patients is insufficient to draw statistically meaningful conclusions, and no formal statistical comparison was performed for this subgroup. These results should therefore be regarded strictly as preliminary, hypothesis-generating observations. Larger prospective cohorts with standardized NAC protocols and systematic pre- and post-treatment tissue sampling are needed to validate whether PRR11 expression has genuine predictive value for NAC response in ER$^+$/HER2-low breast cancer.

Besides its potential prognostic and predictive value, PRR11 expression may also serve as a diagnostic marker for breast cancer. In a study evaluating PRR11 expression in fresh biopsies of both tumoral and adjacent non-tumoral tissues in 70 patients with breast cancer, PRR11 upregulation could distinguish invasive breast cancer tissue from normal tissue with a sensitivity of 92.86%, specificity of 85.71%, AUC of 0.916 and Youden index of 78.6% (27). Additionally, the authors noted an association between PRR11 upregulation and ER/PR positivity, which supports the positive association between ER levels and PRR11 expression observed in the present study.

PRR11 expression is also a potential therapeutic target in other solid tumors, such as non-small cell lung cancer (NSCLC). Zhao (18) reported that targeted depletion of PRR11 expression in lung cancer cells led to cell cycle arrest and apoptotic cell death in patients with NSCLC. In another study, silencing of PRR11 was shown to stimulate autophagy (28). The AKT/mTOR pathway is considered to be one of the primary pathways regulating autophagy (29,30). Inhibition of PRR11 expression may indirectly stimulate autophagy via the AKT/mTOR pathway. The PI3K/AKT/mTOR pathway is a signaling pathway in which gene alterations, such as phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit α mutation, occur during carcinogenesis and follow-up (31). The potential interaction between the PI3K/AKT/mTOR signaling pathway and PRR11 upregulation should therefore also be evaluated to determine potential targets in breast cancer.

In conclusion, the present study revealed a positive association between PRR11 expression and the ER positivity rate in ER$^+$/HER2-low early-stage breast cancer. PRR11 expression did not exhibit a statistically significant association with 5-year DFS or OS in the present cohort, likely reflecting the

favorable OS of this early-stage population and the confounding effect of uniform adjuvant endocrine therapy. Higher PRR11 levels in residual disease after NAC represent a preliminary, hypothesis-generating observation regarding its potential predictive value. PRR11 expression differed according to CerbB2 IHC score in the HER2-low population. Randomized clinical trials and larger prospective studies are needed in this area.

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**Availability of data and materials**

The data generated in the present study may be requested from the corresponding author.

**Authors' contributions**

MD and TB were supervisors. SCI and NB prepared patient pathology specimens from the pathology laboratory archives for analysis. HBE and TB performed the necessary tests for PRR11 expression. AT collected and analyzed the data and wrote the manuscript. MD consulted on the concept analyses during design of the research strategy and writing of the manuscript. MD reviewed and edited the manuscript. AT and TB confirm the authenticity of all the raw data. All authors read and approved the final version of the manuscript.

**Ethics approval and consent to participate**

The present study was approved by the Clinical Research Ethics Committee of Dr. Abdurrahman Yurtaslan Ankara Oncology Training and Research Hospital, Ankara, Turkey (approval no. 2022-12/2195), and was conducted in accordance with the principles of the Declaration of Helsinki. As this was a retrospective study based on archived FFPE tissue samples, the requirement for written informed consent was waived by the ethics committee, and all patient data were anonymized prior to analysis.

**Patient consent for publication**

Not applicable.

**Competing interests**

The authors declare that they have no competing interests.

**References**

1. Lee KM, Guerrero-Zotano AL, Servetto A, Sudhan DR, Lin CC, Formisano L, Jansen VM, González-Ericsson P, Sanders ME, Stricker TP, *et al*: Proline rich 11 (PRR11) overexpression amplifies PI3K signaling and promotes antiestrogen resistance in breast cancer. Nat Commun 11: 5488, 2020.
2. Ji Y, Xie M, Lan H, Zhang Y, Long Y, Weng H, Li D, Cai W, Zhu H, Niu Y, *et al*: PRR11 is a novel gene implicated in cell cycle progression and lung cancer. Int J Biochem Cell Biol 45: 645-656, 2013.
3. Chen J, Yang HM, Zhou HC, Peng RR, Niu ZX and Kang CY: PRR11 and SKA2 promote the proliferation, migration and invasion of esophageal carcinoma cells. Oncol Lett 20: 639-646, 2020.
4. Song Z, Liu W, Xiao Y, Zhang M, Luo Y, Yuan W, Xu Y, Yu G and Hu Y: PRR11 ıs a prognostic marker and potential oncogene in patients with gastric cancer. PLoS One 10: e0128943, 2015.
5. Wang Y, Zhang C, Mai L, Niu Y, Wang Y and Bu Y: PRR11 and SKA2 gene pair is overexpressed and regulated by p53 in breast cancer. BMB Rep 52: 157-162, 2019.
6. Han W and Chen L: PRR11 in malignancies: Biological activities and targeted therapies. Biomolecules 12: 1800, 2022.
7. Wolff AC, Hammond MEH, Allison KH, Harvey BE, Mangu PB, Bartlett JMS, Bilous M, Ellis IO, Fitzgibbons P, Hanna W, *et al*: Human epidermal growth factor receptor 2 testing in breast cancer: American society of clinical oncology/college of American pathologists clinical practice guideline focused update. J Clin Oncol 36: 2105-2122, 2018.
8. Araghi M, Gharebakhshi F, Faramarzi F, Mafi A, Mousavi T, Alimohammadi M and Soleimantabar H: Efficacy and safety of pembrolizumab monotherapy or combined therapy in patients with metastatic triple-negative breast cancer: A systematic review and meta-analysis of randomized controlled trials. Curr Gene Ther 25: 72-88, 2025.
9. Modi S, Jacot W, Yamashita T, Sohn J, Vidal M, Tokunaga E, Tsurutani J, Ueno NT, Prat A, Chae YS, *et al*: Trastuzumab deruxtecan in previously treated HER2-low advanced breast cancer. N Engl J Med 387: 9-20, 2022.
10. Fujii T, Kogawa T, Dong W, Sahin AA, Moulder S, Litton JK, Tripathy D, Iwamoto T, Hunt KK, Pusztai L, *et al*: Revisiting the definition of estrogen receptor positivity in HER2-negative primary breast cancer. Ann Oncol 28: 2420-2428, 2017.
11. Allison KH, Hammond MEH, Dowsett M, McKernin SE, Carey LA, Fitzgibbons PL, Hayes DF, Lakhani SR, Chavez-MacGregor M, Perlmutter J, *et al*: Estrogen and progesterone receptor testing in breast cancer: ASCO/CAP guideline update. J Clin Oncol 38: 1346-1366, 2020.
12. Livak KJ and Schmittgen TD: Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 25: 402-408, 2001.
13. Maltseva DV, Khaustova NA, Fedotov NN, Matveeva EO, Lebedev AE, Shkurnikov MU, Galatenko VV, Schumacher U and Tonevitsky AG: High-throughput identification of reference genes for research and clinical RT-qPCR analysis of breast cancer samples. J Clin Bioinforma 3: 13, 2013.
14. Yau C, Osdoit M, van der Noordaa M, Shad S, Wei J, de Croze D, Hamy AS, Laé M, Reyal F, Sonke GS, *et al*: Residual cancer burden after neoadjuvant chemotherapy and long-term survival outcomes in breast cancer: A multicentre pooled analysis of 5161 patients. Lancet Oncol 23: 149-160, 2022.
15. Wang C, Yu L, Ren X, Wu T, Chen X, Huang Y and Cheng B: The oncogenic potential of PRR11 gene in tongue squamous cell carcinoma cells. J Cancer 10: 2541-2551, 2019.
16. Wei Z, Wang R, Yin X, Zhang L, Lei Y, Zhang Y, Li Y, Wu J, Bu Y, Jin G and Zhang C: PRR11 induces filopodia formation and promotes cell motility via recruiting ARP2/3 complex in non-small cell lung cancer cells. Genes Dis 9: 230-244, 2022.
17. Zhang H, He Z, Qiu L, Wei J, Gong X, Xian M, Chen Z, Cui Y, Fu S, Zhang Z, *et al*: PRR11 promotes cell proliferation by regulating PTTG1 through interacting with E2F1 transcription factor in pan-cancer. Front Mol Biosci 9: 877320, 2022.
18. Zhao Q: RNAi-mediated silencing of praline-rich gene causes growth reduction in human lung cancer cells. Int J Clin Exp Pathol 8: 1760-1767, 2015.
19. Fuentes N and Silveyra P: Estrogen receptor signaling mechanisms. Adv Protein Chem Struct Biol 116: 135-170, 2019.
20. Rosso C and Voutsadakis IA: Characteristics, clinical differences and outcomes of breast cancer patients with negative or low HER2 expression. Clin Breast Cancer 22: 391-397, 2022.
21. Abbasvandi F, Bayat M, Akbari A, Shojaeian F, Zandi A, Rahmani J, Hashemi MO and Akbari ME: Tumor characteristics and survival rate of HER2-low breast cancer patients: A retrospective cohort study. Sci Rep 13: 16719, 2023.

22. Li C, Yuan Q, Deng T, Xu G, Hou J, Zheng L and Wu G: Prognosis difference between HER2-low and HER2-zero breast cancer patients: A systematic review and meta-analysis. Breast Cancer 30: 965-975, 2023.
23. Sakai Y, Ohbayashi C, Yanagita E, Jimbo N, Kajimoto K, Sakuma T, Hirose T, Yoshimura M, Maniwa Y and Itoh T: PRR11 immunoreactivity is a weak prognostic factor in non-mucinous invasive adenocarcinoma of the lung. Pathologica 109: 133-139, 2017.
24. Tan S, Jiang Z, Hou A, Wang J, Zhang J and Dai L: Expression of PRR11 protein and its correlation with pancreatic cancer and effect on survival. Oncol Lett 13: 4117-4122, 2017.
25. Petrelli F, Rea C, Parati MC, Borgonovo K, Ghilardi M, Dottorini L, Luciani A, Ghidini M, Ruatta F, Garrone O and Tomasello G: Prognostic value of HER2-low status in ER+ early breast cancer: A systematic review and meta-analysis. Anticancer Res 43: 4303-4313, 2023.
26. Türkel A, Doğan M, Sertesen E, Karaçin C, Irkkan SC and Ateş Ö: Prognostic factors in non-metastatic HER2 'low' and HER2 'negative' breast cancer: Single institute experience. Wien Klin Wochenschr 136: 340-346, 2024.
27. Anouar Tadlaoui K, Alaoui Sosse S, Benhessou M, El Karroumi M and Ennaji MM: Proline-rich protein 11 over-expression in ınvasive breast carcinoma: A potential diagnosis biomarker. Indian J Gynecol Oncolog 21: 35, 2023.
28. Zhang L, Lei Y, Zhang Y, Li Y, Bu Y, Song F and Zhang C: Silencing of PRR11 suppresses cell proliferation and induces autophagy in NSCLC cells. Genes Dis 5: 158-166, 2017.
29. Heras-Sandoval D, Pérez-Rojas JM, Hernández-Damián J and Pedraza-Chaverri J: The role of PI3K/AKT/mTOR pathway in the modulation of autophagy and the clearance of protein aggregates in neurodegeneration. Cell Signal 26: 2694-2701, 2014.
30. Zhang F, Ma H, Wang ZL, Li WH, Liu H and Zhao YX: The PI3K/AKT/mTOR pathway regulates autophagy to induce apoptosis of alveolar epithelial cells in chronic obstructive pulmonary disease caused by PM2.5 particulate matter. J Int Med Res 48: 300060520927919, 2020.
31. Jiang M, Zhang K, Zhang Z, Zeng X, Huang Z, Qin P, Xie Z, Cai X, Ashrafizadeh M, Tian Y and Wei R: PI3K/AKT/mTOR axis in cancer: From pathogenesis to treatment. MedComm (2020) 6: e70295, 2025.

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