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Title: Diagnostic, prognostic and treatment response of perilipin1 gene in breast cancer

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Abstract

Background: Genetic alterations in the perilipin (PLIN) family genes (*PLIN1 to PLIN5*) were infrequent in breast cancer (BC) where enhanced levels of *PLIN1, PLIN3-5* were observed in the luminal A and luminal B subgroups, whereas increased *PLIN2* expression was observed in the HER2-enriched and basal-like subgroups. However, the predictive value of *PLIN1* for BC patient outcomes remains uncertain. In the present study, we aim to investigate the diagnostic, prognostic and treatment response roles of the *PLIN1* gene expression in BC.

Methods: We obtained microarray BC trancriptomic data of 320 tumor (T) and 62 normal (N) breast samples from five GEO data-series; GSE7904 (38T:7N), GSE42568 (101T: 15N), GSE26910 (6T:6N), GSE45827 (144T:7N), and GSE10810 (31T:27N). The Welch *t* test was used to analyze the significant differences in gene expression including *PLIN1* with fold change > ±2 and *p*-value <0.05. The expression of *PLIN1* was confirmed by RTqPCR using clinical specimen samples from BC patients. The Kaplan-Meier Plotter was used to assess survival on large independent dataset (31 dataset for relapse-free survival and 14 datasets for overall survival) and significance was determined by calculating hazard ratios (>1) and log-rank *p*-values <0.05. We also assessed the treatment outcomes of endocrine therapy (tamoxifen and aromatase-inhibitors), anti-HER2 therapy (trastuzumab and lapatinib), and chemotherapy (taxane, anthracycline, and ixabepilone) using robust statistical methods and correlated with *PLIN1* gene expression.

Results: We identified significantly reduced expression of PLIN1 (FC = -30.76, *p* value = 2.183e-24) in BC samples compared with normal controls. Our qPCR result confirmed the microarray expression pattern of *PLIN1* in BC. Survival analysis revealed *PLIN1* to be a moderately important prognostic biomarker. Our findings highlight the effectiveness of trastuzumab and anthracycline in classifying treatment responses, supported by Mann-Whitney tests indicating statistical significance in gene expression differences between responders and non-responders.

Conclusion: In conclusion, our findings indicate that *PLIN1* is one of the most down-regulated genes and a moderately important biomarker in BC for prognostic purposes. *PLIN1* was a good indicator of trastuzumab and anthracycline treatment responses in BC.

Keywords: Breast cancer, *PLIN1* gene, Gene Expression, Welch test, Kaplan-Meier survival plot, Lancaster, weighted Z and wFisher's method

Abbreviations:

PLIN1: Perilipin 1
BC: Breast cancer
DEGs: Differentially expressed genes
RTqPCR: Real-time quantitative Polymerase chain reaction
GEO: Gene Expression Omnibus
KM: Kaplan and Meier

1. Introduction

Breast cancer (BC) is most common among women, and its global burden is on the rise (Ramaswamy et al., 2001; Karim et al., 2022), and predicted to cross 3 million new cases with >1 million fatalities by the year 2040 (Arnold et al., 2022; Sung et al., 2021). While the majority of early-detected breast tumors are benign and treatable with surgery, approximately 25% of BC tumors exhibit aggressive nature and rapid spread (Cowin et al., 2005). So, it is essential to

understand the underlying molecular mechanisms and discover new biomarkers and targets for cancer diagnosis, prognosis and therapeutics (Iqbal et al., 2023).

Hundreds of differentially expressed key genes such as *BRCA1, BRCA2, PTEN*, *P53*, *KRAS*, and *BRAF* have been reported for BC, but there are also few less explored genes detected in whole gene expression analysis that play a significant role in BC. However, the prognostic value of perilipin *(PLIN)* family members in BC patients remains uncertain. The *PLIN* family genes (*PLIN1-PLIN5*) primarily involved in the formation and degradation of lipid droplets (LDs) but also play significant roles in the development and progression of BC (Zhang et al., 2021). This study aims to evaluate and validate the diagnostic, prognostic, and therapeutic response importance of the *PLIN1* gene in BC.

Statistical analysis using popular student *t*-test faces limitation. It is notably beneficial when comparing two samples with disparate variances and potentially varying sample sizes (Ruxton, 2006; Derrick & White, 2016). It might yield biased results for groups having different variances, because of its underlying assumptions of normality and homoscedasticity (homogeneity of variance), which can lead to unsound and unreliable mathematical inferences (Erceg-Hurn and Mirosevich, 2008). Assumptions of the student's *t*-test require attention, checking and correction when violated. We applied Welch's *t*-test, due to its robust capability (Baguley., 2012; Delacre et al., 2017). Additionally, a Mann-Whitney U test was employed as an additional measure to cross-validate the significant expression of the *PLIN1* gene in breast cancer. This non-parametric statistical test was chosen due to the data's lack of conformity to the assumptions of normality or homoscedasticity.

Survival analysis and prediction of prognosis is a key utility of confirmed differentially expressed *PLIN1*gene in BC. The survival function (probability ranging from one to zero) represents the likelihood that the patients will survive for a minimum specified duration, and it progressively decreases over time. Kaplan-Meier estimator (Kaplan-Meier Plotter) is the most common survival technique for gene expression among semi-parametric (Cox-proportional hazards method), parametric (Weibull and exponential models method), and nonparametric (Kaplan Meier product limit approach) (Emmert-Streib & Dehmer, 2019; Lánczky & Győrffy, 2021). In meta-analysis, data from various studies were combined by robust statistical methods/algorithms ("wFisher," "Lancaster," and "weighted z-method") to derive a single *p* value by combining *p* values of independent cohorts. Weights were assigned to each individual *p*-value according to the sample size (wFisher method), as per degrees of freedom (Lancaster method) and Z transformation (Weighted Z-method) (Yoon et al. 2021).

Determining the predictive role of *PNIL1* by anticipating the response to specific anticancer treatments in BC holds significant importance in tailoring systemic therapy or personalized medicine. It is possible to make therapeutic decisions of selecting hormonal, targeted or chemotherapy by the presence/absence of *PLIN1*, predictive biomarkers (Fekete & Győrffy, 2019). The selection of therapy depends on the tumor's molecular/pathological characteristics and patient's expected survival outcome (prognosis). We utilized the ROC Plotter online tool (https://www.rocplot.com/) to comprehensively investigate the correlation between the expression of the *PLIN1* gene and assess the response to different therapies including endocrine therapy (tamoxifen and aromatase inhibitor), anti-HER2 therapy (trastuzumab and lapatinib), and chemotherapy (taxane, anthracycline, and ixabepilone) in BC (Fekete & Győrffy, 2019).

2. Materials and Methods

2.1 Gene expression microarray data

We retrieved raw gene expression data in dot CEL files format from GEO (Gene Expression Omnibus) database at NCBI, a public repository for microarray and next-generation sequencing data (https://www.ncbi.nlm.nih.gov/geo/). A total of 320 BC tumor samples and 62 control samples were obtained from GSE7904, GSE42568, GSE26910, GSE45827 and GSE10810 data series.

2.2 Identification of differentially expressed genes

2.2.1 Welch Satterthwaite *t*-test: We applied Welch Satterthwaite *t*-test using the formula below to compare the mean and detect the significant difference between BC and control:

$$\omega(t) = \frac{\Delta\bar{X}}{s_{\Delta\bar{X}}} = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{s^2_{\bar{X}_1} + s^2_{\bar{X}_2}}} . \quad (1)$$

$$s_{\bar{X}_i} = \frac{s_i}{\sqrt{Ni}} . \quad (2)$$

$\bar{X}_i =$ i^{th} sample means

$s_{\bar{X}_i}$ $= i^{th}$ standard error, for a particular example size and standard deviation

The Welch degrees of freedom $\omega(\nu)$ was calculated by using the Welch-Satterthwaite equation below:

$$\omega(\nu) \approx \frac{\left(\frac{s_1^2}{N_1} + \frac{s_2^2}{N_2}\right)^2}{\frac{s_1^4}{N_1^2\omega(\nu_1)} + \frac{s_2^4}{N_2^2\omega(\nu_2)}} . \quad (3)$$

If $N_1 = N_2$

$$\omega(\nu) \approx \frac{s^4_{\Delta\bar{X}}}{\omega(\nu_1)^{-1}s^4_{\bar{X}_1} + \omega(\nu_2)^{-1}s^4_{\bar{X}_2}} . \quad (4)$$

Where $\omega(\nu_i) = N_i - 1$ was the degree of freedom.

2.2.2 Mann–Whitney U test: It is a nonparametric test and was applied as an additional measure for cross-validation: It has a null hypothesis: for randomly selected values x and y from tumor and control samples respectively, the probability of x being greater than y is equal to the probability of y being greater than x. *p* value of statistical significance was 0.05.

2.3 Real-time quantitative PCR

We used RTqPCR assay with Applied Biosystems StepOnePlus Real-Time PCR instrument (ThermoFisher Scientific, USA) to validate the expression of *PLIN1* gene. PowerUp™ SYBR™ Green Master Mix with reference (GAPDH1 was used for Quantification. Comparative Ct (ΔΔCt) method was used for quantitative gene expression based on initial Ct values calculated by DataAssist™ Software. Additionally, RNA-seq results at UALCAN portal (https://ualcan.path.uab.edu/index.html) were used to confirm the expression pattern of *PLIN1* at an independent bigger cohort of TCGA dataset.

2.4 Survival Study

It was used to assess time-to-event data, such as the time until death or the amount of time needed for a particular event to occur. Survival analysis was conducted by determining the association between gene expression data and patient survival or the development of a disease. It provides insightful information behind the advancement of BC patients and their outcomes (prognosis), and statistical model for diagnostic and therapeutic strategies.

2.4.1 Hazard function and hazard ratio: The hazard function, which represents the instantaneous death rate, was defined as the likelihood that a person will pass away at a specific moment, presuming that person has survived up to that point. The survival function can be shown as

$$h(t) = \lim_{\Delta t \to 0} \frac{P[death(t, t+\Delta t)]}{\Delta t} . \quad (5)$$

where death (*x*,*y*) refers to a person's passing between the ages of *x* and *y* within that time period.

$$H(t) = -log_e s(t)\,. \quad (6)$$

HR=1 (no risk difference between the groups); and HR>1 indicating some risk.

2.4.2 Kaplan-Meier method: This method determines the probability of passing away at a specified moment assuming the person has so far survived using following mathematical formulation of the KM estimator (Kaplan and Meier, 1958):

$$SF(\text{Survival Function})\ at\ t = \prod_{i=t_i<t} \frac{n_i - d_i}{n_i} = \prod_{i=t_i<t} (1 - d_i/n_i)\,. \quad (7)$$

When two events occur, the survival curve does not change, in t_i and t_i+1.
recursive formula

$$SF\ at\ t_j = [\frac{n_{j-1} - d_{j-1}}{n_{j-1}}\ ] \text{ multiply by (SF) at } t_{j-2}\,. \quad (8)$$

"Kaplan-Meier Plotter" was used (https://kmplot.com/analysis/) for survival analysis (Emmert-Streib & Dehmer, 2019; Lánczky & Győrffy, 2021)

2.5 Response to therapy

To rigorously assess the connection between gene expression and therapy response, we employed robust statistical methods: receiver operating characteristics (ROC) analysis and Mann-Whitney tests. The online platform ROC plotter (https://www.rocplot.com/) was used to validate the relationship between *PLIN1* gene expression and the response to various therapies typically used in breast cancer including endocrine therapy drugs (tamoxifen and aromatase-inhibitors), anti-HER2 therapy drugs (trastuzumab and lapatinib), and chemotherapy drugs (taxane, anthracycline, and ixabepilone).

2.5.1 Receiver operating characteristics (ROC), area under curve (AUC) analysis: The ROC curve was the plot of the true positive rate against the false positive rate at each threshold setting, while AUC showed how well the test separated the two groups. A large area under the ROC curve predicted better treatment response. AUC values above 0.6 was acceptable, AUC values between 0.6 – 0.7 indicated clinically potential cancer biomarker, AUC values between 0.7 – 0.8 indicated high quality cancer biomarker, and AUC values above 0.8 referred to blockbuster biomarker.

2.5.2 Mann-Whitney U Test for response to therapy: The Mann-Whitney U test was a rank-based non-parametric test used here to determine if there are differences between two groups. Characteristics of the groups are usually presented by employing a box-and-whisker plot and *p*-value < 0.05 was considered significant (Fekete & Győrffy, 2019).

3. Results

3.1 Microarray data and samples: We retrieved expression data from five datasets: GSE7904, GSE42568, GSE26910, GSE45827, and GSE10810, to analyze the expression of *PLIN1* in breast cancer. A total of 320 tumor samples and 62 control samples were obtained from GSE7904 (38 tumor samples and 7 control samples), GSE42568 (101 tumor samples and 15 control samples), GSE26910 (6 tumor samples and 6 control samples), GSE45827 (144 tumor samples and 7 control samples), and GSE10810 (31 tumor samples and 27 control samples).

3.2 Expression profiling and statistical tests: We found notably down expression of *PLIN1* gene in individual data series with fold change (FC) of -6.91, -53.93, -2.17, -35.46 and -24.63 for GSE7904, GSE42568, GSE26910, GSE45827, GSE10810 respectively, and FC = -30.76 for combined data of 320 tumors and 62 control samples. The Tukey Fence method used for outlier detection, suggested that the tumor dataset contains 11 (3.44%) potential outliers, while the control dataset contains 10 (16.13%) potential outliers. The Shapiro-Wilk test yielded W= 0.89 with *p*-value of 8.611e-14 for the tumor dataset, and W= 0.74 with *p*-value of 5.958e-09 for the control

dataset, indicating significant deviation from a normal distribution. The Kolmogorov-Smirnov test resulted in D =1 with *p*-values of 2.2e-16 for the tumor dataset and D =1 with *p*-values of 7.772e-16 for the control dataset, demonstrating significant deviations from the theoretical distribution under test, such as a normal distribution. Histograms and QQ plots validated the normality in our tumor and control datasets (**Figure 1A**). These visualizations indicated that tumor sample was somewhat close to a normal distribution. Due to the non-normality observed, we applied the Mann-Whitney U test (a non-parametric test) along with the Welch *t*-test (a parametric test) using WRS2 library in R. Here, Welch *t*-test, *t*-value = -15.63 with *p*-value of 2.183e-24, felled outside the region of acceptance (±1.99) at a 95% confidence level, and the null hypothesis (***Ho***) was rejected.

3.3 Validation by RTqPCR: Validation of differentially expressed gene was performed by qPCR. *PLIN1* was significantly under-expressed (Rq = 0.03, FC= - 29.22, *p*-value 1.55667E-^{10}) and qPCR confirmed the microarray expression data in BC. Additionally, RNA-seq results also confirmed the significant under-expression of *PLIN1* (transcript per million 2.267 and *p* value 1.1102E^{-16}) on a bigger cohort (**Figure 1B and 1C**).

3.4 Prognostic importance of *PLIN1* gene in breast cancer: We conducted a Kaplan-Meier analysis for prognostic purposes to assess the impact of *PLIN1* expression on the survival outcomes of BC patients, including relapse-free survival (RFS), overall survival (OS), distant metastasis-free survival (DMFS), and progression-free survival (PFS). The study unveiled *PLIN1* as a potential and significant biomarker with therapeutic and prognostic using Benjamini-Hochberg technique and the log-rank *p*-value with 75% relevance (**Table 1** and **Figure 2A**).

We evaluated the impact of *PLIN1* gene expression RFS within various BC subtypes including ER array, ER IHC, PR, Lymph node status, HER2 status, luminal androgen receptor, Pietenpol subtypes (basal-like 1 & 2), immunomodulatory, StGallen subtypes (basal-like, HER2+, luminal A, and luminal B) mesenchymal, and mesenchymal stem-like, and observed four out of twenty cases exhibited significant associations, indicating moderate importance of the *PLIN1* gene in BC (**Supple Table 1** and **Figure 2B**). In Figure 2B, we have shown only the RFS ER status, indicating Array ER positivity and negativity, as well as RFS ER status determined through IHC positive and negative cases. We conducted prognostic survival analysis for OS under various BC subtypes and observed five out of the twenty samples exhibited significant results, indicating moderate importance of the *PLIN1* gene in BC (**Supple Table 1** and **Figure 3A**). In Figure 3A, we have shown only the OS ER status, indicating Array ER positivity and negativity, as well as OS ER status determined through IHC positive and negative cases. Moreover, we conducted additional assessments of the *PLIN1* gene's impact on RFS using 31 independent GEO series and OS using 14 independent GEO series in BC, and observed 2 out of 31 GEO series exhibited significant associations with RFS (**Supple Table 2** and **Figure 3B**) and 1 out of 14 GEO series exhibited significant associations with OS (**Supple Table 2** and **Figure 4A**), confirming the significant and moderately predictive relevance of the *PLIN1* gene in BC. In Figure 3B, only RFS for the independent data series GSE1456, GSE2034, GSE2603, and GSE2990 are presented. In Figure 4A, only OS for the independent data series GSE1456, GSE3494, GSE7390, and GSE16446 are shown. We utilized *p*-value integration techniques (wFisher, Lancaster, and Weighted Z-Methods) within the R library (metapro) to combine the individual *p*-values from 31 independent cohorts of RFS. The combined *p*-values were 0.0045 for Weighted Z-method, 0.0011 for Lancaster method and 0.0004 for wFisher method, strongly indicated a significant influence of *PLIN1* gene expression on the survival of BC patients. Similarly, the meta-analysis conducted OS data from 14 independent cohorts, showed combined *p*- value of 0.0024 for the Weighted Z-method, 0.00209 for Lancaster method, and 0.0015 for the wFisher method, strongly indicated a significant

influence of *PLIN1* gene expression on the OS of BC patients. mRNA expression of the *PLIN1* gene (RNA-seq) was used to conduct an evaluation of OS for BC subtypes and observed four out of thirteen cases exhibited significant associations, indicated moderate prognostic importance of the *PLIN1* gene in BC (**Supple Table 3** and **Figure 4B**). In Figure 4B, OS for only lymph node status for mRNA (RNA Seq) were shown, with positive and negative cases, as well as HER2 status for mRNA (RNA Seq), highlighting both positive and negative instances.

3.5 Treatment response to various therapies: We examined the relationship between *PLIN1* gene expression and the response to various therapies in BC. Our investigation encompasses a wide spectrum of systematic therapies typically used in BC treatment such as tamoxifen and aromatase-inhibitors under endocrine therapy, trastuzumab and lapatinib under targeted anti-HER2 therapy, and taxane, anthracycline, and ixabepilone as chemotherapy. We employed two robust statistical methods: ROC analysis and Mann-Whitney tests and constructed box plots to illustrate the distinction between responders and non-responders across all the therapy types under investigation (**Figures 5**). The key quantitative results of AUC, ROC, and Mann-Whitney test for *PLIN1* gene had been recorded as treatment response outcomes (**Tables 2**). Here, anti-HER2 therapy with trastuzumab and chemotherapy with anthracycline exhibited exceptional efficacy in terms of the gene's ability to classify treatment responses, suggesting potential clinical utility for BC. Mann-Whitney U test had also revealed that the differences in *PLIN1* gene expression between responders and non-responders in these two treatment groups were statistically significant (p-values <0.05), established significant association between *PLIN1* gene expression and the effectiveness of these specific treatments.

4. DISCUSSION:

The *PLIN1* gene, primarily expressed in white adipose tissues, plays a role in hormone-induced lipolysis and large lipid droplets formation (Sztalryd & Kimmel, 2014). Studies have highlighted elevated *PLIN1* expression in liposarcoma (a cancerous tumor of lipoblast) and its absence in lipoma (benign soft tissue lump) (Straub et al., 2019). The role of *PLIN1* expression in BC is variable and under-investigation. The present study extensively examined the expression patterns and prognostic implications of the *PLIN1* gene in BC.

Applying a trustworthy and accurate statistical approach for identifying substantially expressed genes is a crucial component of high-throughput microarray data processing. The variances of two sets of data cannot be expected to be homogenous with standard deviation ratio of 1:1 in statistical testing under actual conditions (Erceg-Hurn and Mirosevich 2008). Variance ratios might range between 1.1 and 1.2 in the majority of studies, and their reasons are still not fully understood. We, therefore, used Welch's *t*-test to measure the expression of the *PLIN1* gene in BC because it does not require to meet homoscedasticity requirement. Our results revealed significantly lower expression of *PLIN1* (FC -30.76 and p value $2.15848E^{-10}$) in BC tissues compared to normal. Earlier studies have also reported low expression of *PLIN1* in BC on sample size supporting out result (Karim et al., 2023; Zhou et al., 2016; Kim et al., 2015). Survival analysis indicated that *PLIN1* expression was associated with relapse free survival (RFS) and overall survival (OS) in BC patients. Lower expression of *PLIN1* indicated poor prognosis while high expression was associated with longer survival of BC patients (Zhang et al., 2021; Zhou et al., 2016). Conversely, Jung et al. linked high *PLIN1* expression to shorter overall survival in metastatic breast cancer (Jung et al., 2015). Notably, Zhou et al. observed low *PLIN1* expression predicting poorer metastatic relapse-free survival in ER-enriched and luminal-A subtypes (Zhou et al., 2016).

Nevertheless, further studies are necessary to validate existing results (Zhou et al., 2016; Jung et al., 2015).

Survival analysis and predicting prognosis with *PLIN1* expression was another dimension of this study. Unfortunately, censoring where patients either die from a disease other than the disease of interest or are lost to follow-up, can affect the survival curves if many people are censored at one time point (Leung et al., 1997). Usually, the average or median times (follow-up) approach was applied to address censoring issue (Machin et al. 2007). For survival analysis either a non-parametric approach where no assumptions are required on the hazard/survival rate or a parametric techniques for determining the variables effect on hazard/survival rate, such as demographic parameters, illness type, and therapy received are used such as Kaplan-Meier (KM) plotter and the Cox-proportional hazards model (CPHM) (Tseng et al., 2012; Evangelou & Ioannidis, 2013). CPHM is preferred to handle variable effects while KM plotter is preferred while analyzing time-to-event data in the field of cancer to estimate curves, and other crucial tables like overall comparisons (Etikan, 2017). We employed the KM plot as a confirmatory test, considering the positive or negative status of patient's ER, PR, and LN to give the investigation a novel perspective.

Meta-analysis on several independent cohorts is done by statistical methods using "metapro" package of R to boost statistical power (Yoon et al., 2021; Whitlock, 2005). The weighted version of the Fisher's approach (wFisher) has stronger power as it assign specific weight to specific experimental circumstances or genetic differences used frequently in analyzing high-throughput microarray and RNA-seq data (Yoon et al., 2021).

Here wFisher, Lancaster, and Weighted Z-method were used for meta-analysis, and our findings clearly suggest the *PLIN1* expression considerably impacted on BC patient's survival.

The primary challenge in BC management is selection of drug(s), initiation of treatment and predicting therapeutic outcome, and this clinical decision is a crucial turning point that heavily depends on accurate and timely diagnosis. Presently, a fusion approach using conventional clinicopathological factors and molecular biomarkers, encompassing single-gene tests (ER, PR, HER2) and/or specialized gene signatures are used to improve diagnosis accuracy (Iqbal et al, 2023; Mirza et al., 2023; Karim et al, 2016; Merdad et al, 2015; Merdad et al, 2014).

Receiver operating characteristics (ROC) plotter and Mann-Whitney tests was used to extensively explore the response of various therapies in BC in relation to *PLIN1* expression (Fekete & Győrffy, 2019). We found two drugs i.e trastuzumab and anthracycline exhibiting exceptional efficacy in terms of the *PLIN1* gene's ability to classify treatment responses when investigated a wide spectrum of BC therapies including endocrine therapy with tamoxifen and aromatase inhibitors, anti-HER2 therapy with trastuzumab and lapatinib, and chemotherapy with taxane, anthracycline, and ixabepilone. Additionally, Mann-Whitney U test has revealed statistically significant differences in gene expression between responders and non-responders in trastuzumab and anthracycline treatment groups. Consequently, it can be inferred that the *PLIN1* gene holds potential biomarker of intermediate significance in terms of BC.

Further, a breast cancer diagnosis, treatments and symptom management exerts a profound and enduring psychological impact on individuals. A follow-up of elevated rates of depression and anxiety persisting up to five years post-diagnosis is recommended to manage the emotional strain in BC patients (Blaes et al., 2023). The psychological effects on cancer patients vary throughout the diagnostic and treatment phases. Following a breast cancer diagnosis, individuals typically undergo elevated negative emotions, psychological distress, anxiety, depression, shock, denial, and subsequent stress and worry (Fortin et al., 2021; Compas & Luecken, 2002; Martino et al.,

2021; Yang et al., 2017). Ignoring this aspect risks crucial elements influencing both the mental and physical well-being of BC patients.

5. CONCLUSION:

The *PLIN1* gene had significantly lower expression in BC tissues compared to normal. Survival analysis revealed the moderate importance of *PLIN1* in predicting prognosis. Response to trastuzumab and anthracycline treatment showed an intermediate significance of *PLIN1* in BC.

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Data Availability: All the data is available in public domain (https://www.ncbi.nlm.nih.gov/geo/). Authors will provide additional information upon reasonable request.

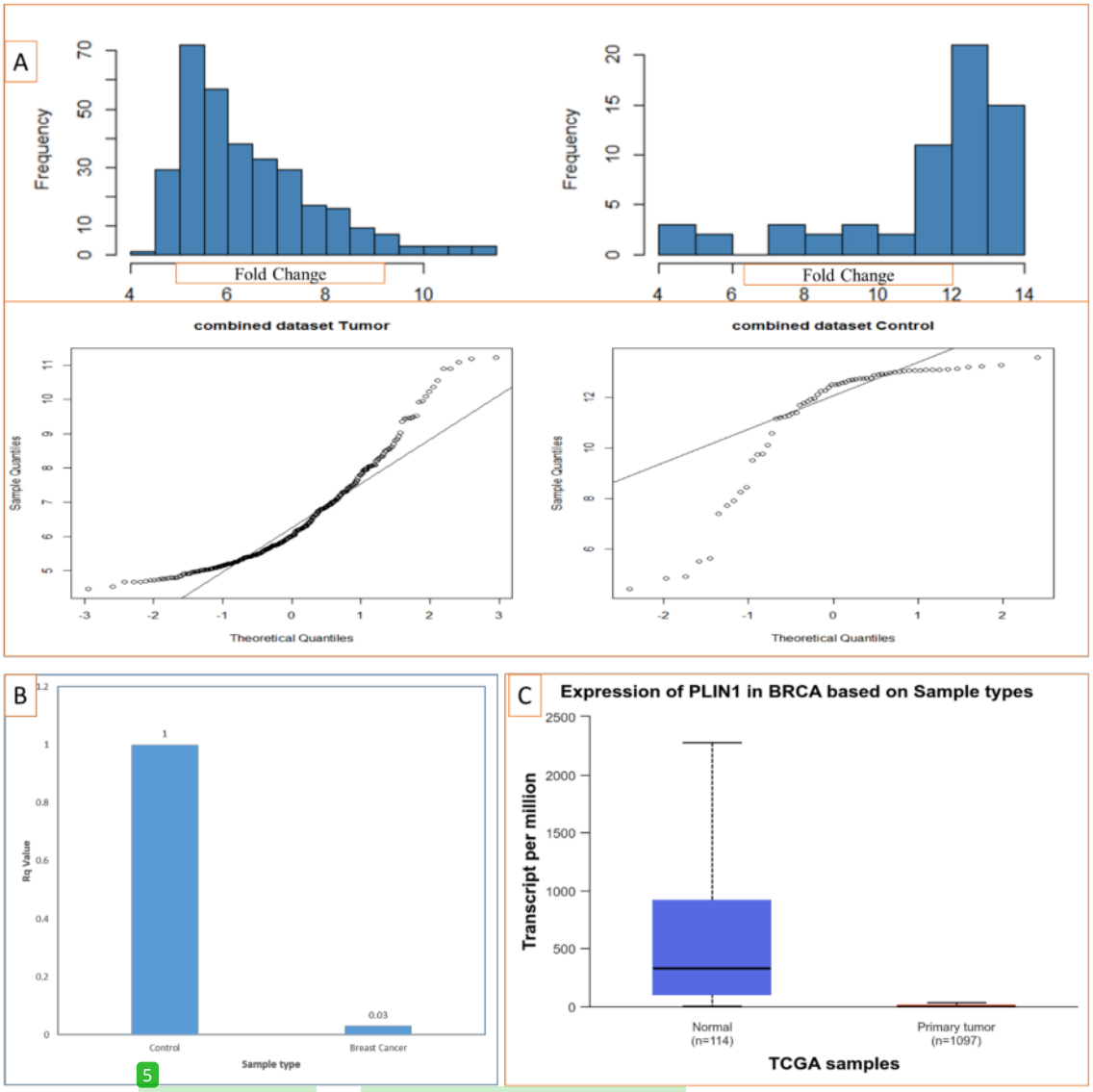

Figure 1: **A**. Normality check for the assumption of Welch *t*-test for the tumor and control dataset using Histogram and QQ (Quantile-Quantile) plots, **B.** RTqPCR result showing down-expression of *PLIN1* in breast cancer. **C.** RNA-Seq results from TCGA database at UALCAN portal also showing under-expression of PLIN1 in breast cancer

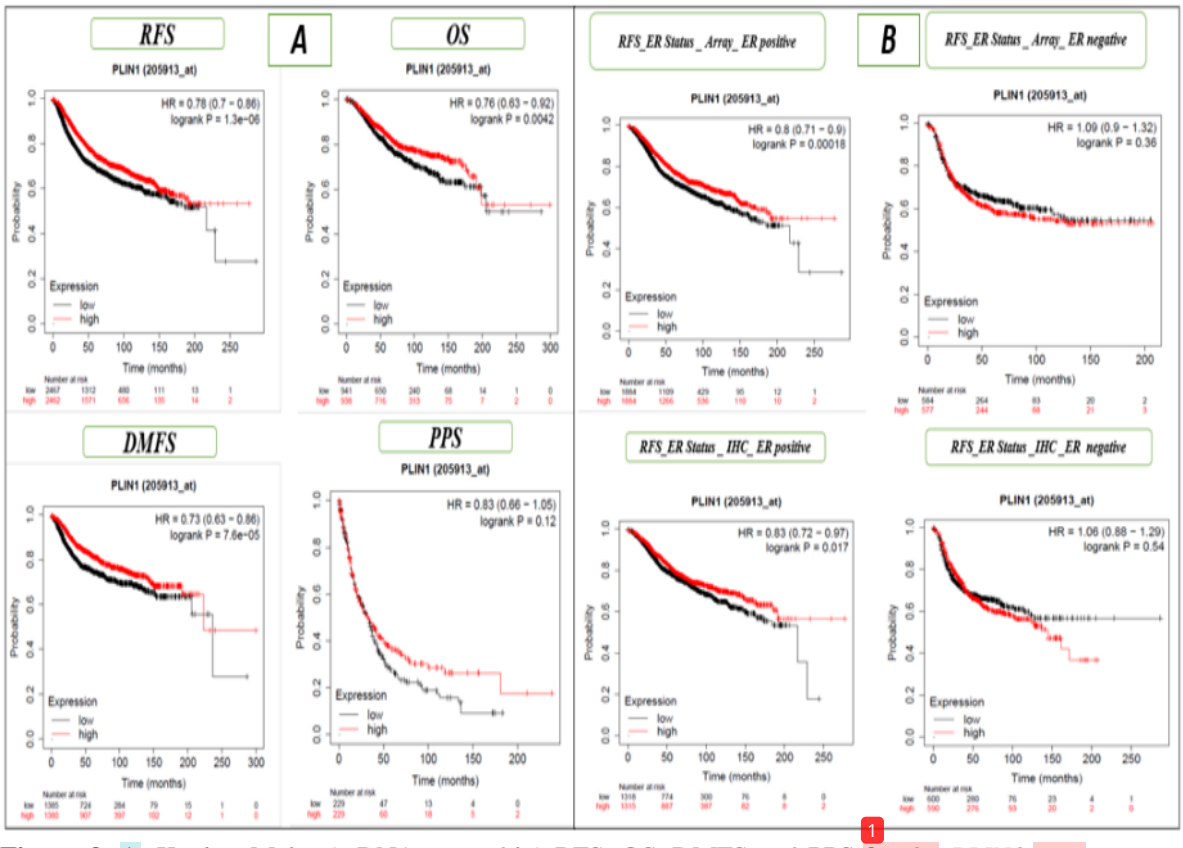

Figure 2: **A.** Kaplan–Meier (mRNA-gene chip) RFS, OS, DMFS and PPS for the *PLIN1* gene without any restriction to subtypes, **B.** Kaplan–Meier (mRNA-gene chip) relapse-free survival for ER array and IHC ER status for the *PLIN1* gene

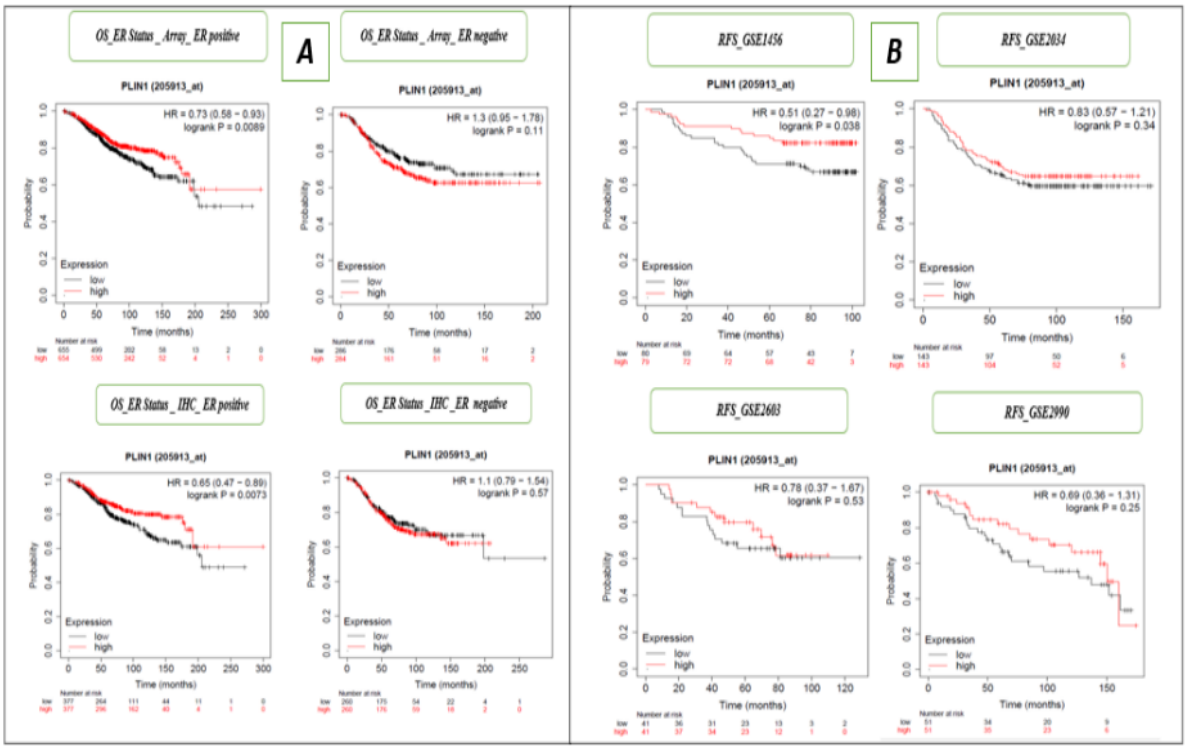

Figure 3: **A**. Kaplan–Meier overall survival (mRNA-gene chip) array and IHC ER status for the *PLIN1* gene, **B.** Kaplan–Meier relapse-free survival (mRNA-gene chip) for individual datasets (GSE1456, GSE2034, GSE2603 and GSE2990) for the *PLIN1* gene

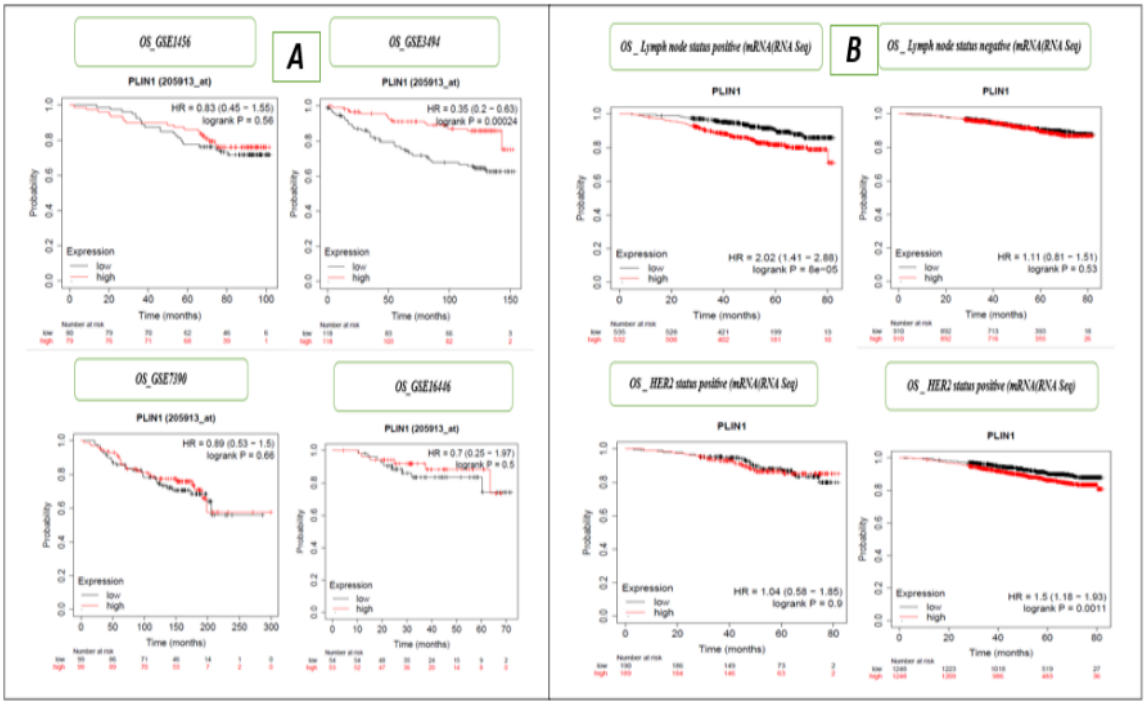

Figure 4: **A.** Kaplan–Meier overall survival (mRNA-gene chip) or individual datasets (GSE1456, GSE3494, GSE7390 and GSE16446) for the *PLIN1* gene, **B.** Kaplan–Meier overall survival (mRNA-RNA Seq) for lymph node and HER2 status for the *PLIN1* gene

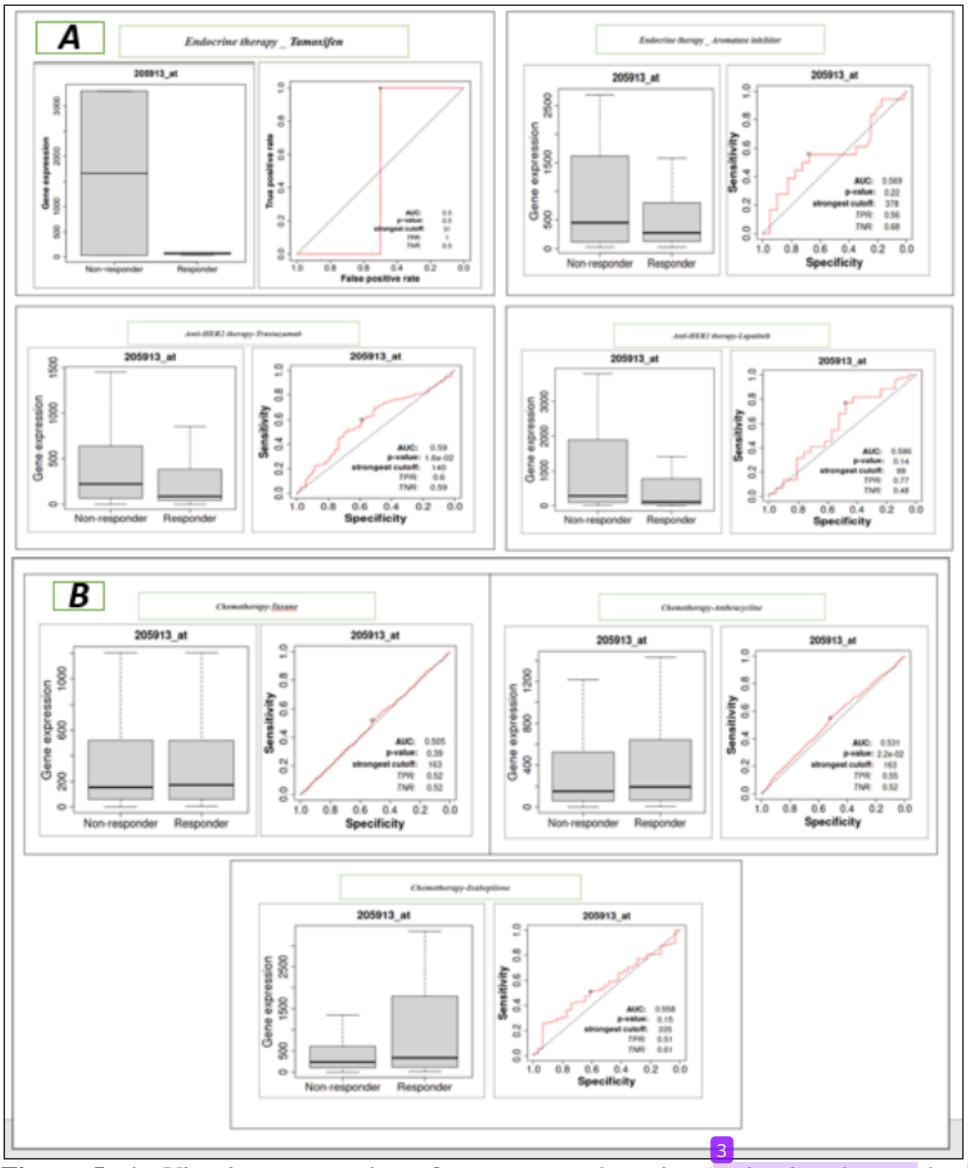

Figure 5: **A.** Visual representation of response to therapies (endocrine therapy by tamoxifen, aromatase inhibitor, and anti-HER2 therapy by trastuzumab and lapatinib) for number of responders, number of non-responders, AUC value, ROC *p*-value and Mann-Whitney test *p*-value, **B.** Visual representation of response to chemotherapies (Taxane, Anthracycline and Ixabepilone) for number of responders, number of non-responders, AUC value, ROC *p*-value and Mann-Whitney test *p*-value.

Table 1: Survival analysis (mRNA-gene chip) using Kaplan–Meier plotter for RFS, OS, DMFS and PPS for the *PLIN1* gene without any restriction to subtypes. Hazard ratio with 95% confidence intervals, log rank *p* value and adjusted *p* value (Benjamini-Hochberg method) was deciding the significance.

mRNA (gene chip): *PLIN1* Probes ID:205913 without restriction to subtype							
Survival Type*	HR	CI	Log rank *p* value	Rank	Adjusted significance level (rank/m)*α(0.05)	Log rank *p* value less than adjusted significance level	Decision
RFS	0.78	0.7 - 0.86	0.000001	1	0.013	TRUE	Significant
OS	0.76	0.63-0.92	0.0042	2	0.025	TRUE	Significant
DMFS	0.73	0.63-0.86	0.000076	3	0.038	TRUE	Significant
PPS	0.83	0.66-1.05	0.1243	4	0.05	FALSE	Insignificant

Survival types: relapse free survival (RFS), overall survival (OS), distant metastasis free survival (DMFS) and progression free survival (PPS)

Table 2: Response to therapies for endocrine therapy, anti-HER2 therapy and chemotherapy for number of responders, number of non-responders, AUC value, *p*-value for ROC and Mann-Whitney test

Inputs parameters	**Endocrine therapy**		**Anti-HER2 therapy**		**Chemotherapy**		
	Tamoxifen	**Aromatase inhibitor**	**Trastuzumab**	**Lapatinib**	**Taxane**	**Ixabepilone**	**Anthracycline**
Number of responders	5	40	87	21	371	105	528
Number of non-responders	2	18	99	44	842	31	1098
AUC pathological response	0.5	0.569	0.59	0.586	0.505	0.558	0.531
ROC *p*-value	0.5	0.22	0.016	0.14	0.39	0.15	0.022
Mann-Whitney test *p*-value	1	0.41	0.034	0.27	0.79	0.33	0.044

Supplementary Table 1: Relapse free survival and overall survival analyses for the *Perilipin 1* using mRNA-gene chip data with subtype boundaries, HR with 95% CI, log rank *p*-value, Benjamini-Hochberg based adjusted significance level and decision-making based on the *p*-value

Survival analysis with subtype boundaries	Hazard ratio		Confidence intervals		Log rank *p*-value		Rank		Adjusted significance level: (rank/m)*α(0.05)		Decision #	
	RFS	**OS**	**RFS**	**OS**	**RFS**	**OS**	**RFS**	**OS**	**RFS**	**OS**	**RFS**	**OS**
HER2 status: array negative	0.73	0.7	0.65-0.82	0.56-0.87	0.000000047	0.0015	1	1	0.003	0.003	S	S
ER Status: array-ER positive	0.8	0.62	0.71-0.9	0.45-0.86	0.0001	0.0036	2	2	0.005	0.005	S	S
Subtype: StGallen luminal A	0.77	4.68	0.65-0.9	1.41-15.53	0.0014	0.0064	3	3	0.008	0.008	S	S
Lymph node status: positive	0.78	0.65	0.66-0.93	0.47-0.89	0.0042	0.0073	4	4	0.01	0.01	S	S
ER Status: IHC-ER positive	0.83	0.73	0.72-0.97	0.58 - 0.93	0.017	0.0089	5	5	0.013	0.013	I	S
Lymph node status: negative	0.83	0.68	0.71-0.98	0.48-0.96	0.0261	0.029	6	6	0.015	0.015	I	I
Subtype - StGallen HER2+	1.4	1.3	0.98-2.01	0.95 - 1.78	0.0629	0.1055	7	7	0.018	0.018	I	I
HER2 status: array positive	1.2	0.75	0.96-1.49	0.53-1.07	0.103	0.1119	8	8	0.02	0.02	I	I
Pietenpol subtype: basal-like 1	1.4	1.53	0.91-2.15	0.86-2.72	0.1246	0.1494	9	9	0.023	0.023	I	I
Pietenpol subtype: mesenchymal	1.32	1.6	0.89-1.96	0.83-3.12	0.1701	0.1588	10	10	0.025	0.025	I	I
Subtype: StGallen luminal B	0.9	1.72	0.75-1.07	0.79-3.75	0.224	0.1683	11	11	0.028	0.028	I	I
ER Status: array-ER negative	1.09	1.2	0.9 - 1.32	0.82-1.75	0.3586	0.3524	12	12	0.03	0.03	I	I
ER Status: IHC-ER negative	1.06	1.18	0.88-1.29	0.82-1.7	0.543	0.3621	13	13	0.033	0.033	I	I
Subtype: StGallen_basal	1.06	1.1	0.85-1.33	0.79-1.54	0.5811	0.5733	14	14	0.035	0.035	I	I
PR status: IHC-PR positive	1.06	1.11	0.79-1.41	0.68-1.81	0.7113	0.6642	15	15	0.038	0.038	I	I
Pietenpol subtype: basal-like 2	0.91	1.24	0.5-1.67	0.46-3.33	0.7663	0.6749	16	16	0.04	0.04	I	I
Pietenpol subtype: mesenchymal stem-like	0.94	0.93	0.46-1.9	0.67-1.3	0.8597	0.6839	17	17	0.043	0.043	I	I
Pietenpol subtype: immunomodulatory	1.04	0.89	0.66-1.64	0.49-1.62	0.8607	0.7011	18	18	0.045	0.045	I	I
PR status: IHC-PR negative	0.99	0.91	0.79-1.25	0.44-1.92	0.9261	0.8126	19	19	0.048	0.048	I	I
Pietenpol subtype: luminal androgen receptor	1.01	0.91	0.69-1.48	0.42-2.01	0.9528	0.8244	20	20	0.05	0.05	I	I

Decision based on log rank p value less than adjusted significance level; S = Significant; I = Insignificant

Supplementary Table 2: Relapse free survival and overall survival analyses for the *Perilipin 1* using mRNA-gene chip for independent GEO series with the hazard ratio, 95% confidence intervals, log rank *p* value, Benjamini-Hochberg based adjusted significance level and the decision based on *p* value

Relapse Free Survival							
GEO Series: Accession No.	**Sample Size**	**HR**	**CI**	**Log rank p value**	**Rank**	**Adjusted significance level (rank/m)*α(0.05)**	**Decision**
GSE61304	62	0.14	0.04-0.50	0	1	0.0016	Significant
GSE3494	251	0.51	0.33-0.79	0.002	2	0.0032	Significant
GSE5327	58	0.18	0.04-0.84	0.014	3	0.0048	Insignificant
GSE12093	136	0.32	0.12-0.86	0.018	4	0.0065	Insignificant
GSE17907	54	3.66	1.15-11.63	0.019	5	0.0081	Insignificant
GSE1456	159	0.51	0.27-0.98	0.038	6	0.0097	Insignificant
GSE48390	81	3.17	0.86-11.75	0.068	7	0.0113	Insignificant
GSE42568	121	0.62	0.35-1.1	0.099	8	0.0129	Insignificant
GSE17705	196	0.64	0.37-1.11	0.111	9	0.0145	Insignificant
GSE20685	327	0.74	0.48-1.14	0.175	10	0.0161	Insignificant
GSE25066	507	0.78	0.54-1.14	0.195	11	0.0177	Insignificant
GSE20711	90	1.46	0.77-2.76	0.239	12	0.0194	Insignificant
GSE2990	102	0.69	0.36-1.31	0.252	13	0.021	Insignificant
GSE4611	153	1.5	0.72-3.12	0.272	14	0.0226	Insignificant
GSE19615	115	0.55	0.18-1.64	0.275	15	0.0242	Insignificant
GSE2034	286	0.83	0.57-1.21	0.336	16	0.0258	Insignificant
GSE16446	120	0.69	0.31-1.55	0.367	17	0.0274	Insignificant
GSE46184	74	1.42	0.62-3.23	0.405	18	0.029	Insignificant
GSE45255	139	0.7	0.29-1.7	0.432	19	0.0306	Insignificant
GSE6532	82	1.39	0.55-3.55	0.486	20	0.0323	Insignificant
GSE12276	204	0.91	0.69-1.2	0.502	21	0.0339	Insignificant
GSE26971	276	0.78	0.37-1.63	0.504	22	0.0355	Insignificant
GSE2603	99	0.78	0.37-1.67	0.527	23	0.0371	Insignificant
GSE16391	55	0.76	0.21-2.72	0.676	24	0.0387	Insignificant
GSE7390	198	1.08	0.71-1.64	0.704	25	0.0403	Insignificant
GSE69031	129	1.13	0.6-2.12	0.705	26	0.0419	Insignificant
GSE11121	200	1.1	0.62-1.97	0.742	27	0.0435	Insignificant
GSE31519	67	0.88	0.38-2.05	0.771	28	0.0452	Insignificant
GSE21653	240	1.06	0.67-1.68	0.794	29	0.0468	Insignificant
GSE65194	164	0.84	0.19-3.78	0.825	30	0.0484	Insignificant
GSE9195	77	1.08	0.36-3.22	0.886	31	0.05	Insignificant
Overall Survival							
GSE3494	251	0.35	0.2-0.63	0.0002	1	0.0036	Significant
GSE42568	121	0.49	0.25-0.97	3.60E-02	2	0.0071	Insignificant
GSE48390	81	4.39	0.93-20.68	0.0412	3	0.0107	Insignificant
GSE37946	41	3.97	0.8-19.74	0.0692	4	0.0143	Insignificant
GSE69031	130	1.91	0.89-4.1	0.093	5	0.0179	Insignificant
GSE20685	327	0.74	0.48-1.15	0.18	6	0.0214	Insignificant
GSE58812	107	0.63	0.3-1.32	0.2156	7	0.025	Insignificant

GSE22093	68	1.85	0.61-5.67	0.272	8	0.0286	Insignificant
GSE45255	139	0.69	0.27-1.76	0.4373	9	0.0321	Insignificant
GSE16446	107	0.7	0.25-1.97	0.4963	10	0.0357	Insignificant
GSE1456	159	0.83	0.45-1.55	0.5582	11	0.0393	Insignificant
GSE7390	198	0.89	0.53-1.5	0.6618	12	0.0429	Insignificant
GSE65194	130	1.15	0.45-2.91	0.7721	13	0.0464	Insignificant
GSE20711	90	1.12	0.51-2.46	0.7749	14	0.05	Insignificant

Decision based on log rank p value less than adjusted significance level

Supplementary Table 3: Overall survival analysis using mRNA-RNA-seq with subtype boundaries for the *PLIN1* gene along with the hazard ratio with 95% confidence intervals, log rank *p* value and the decision based on *p* value and adjusted significance level through Benjamini-Hochberg method

Survival Analysis with subtype boundaries	**HR**	**CI**	**Log rank *p* value**	**Rank**	**Adjusted significance level(rank/m)*α(0.05)**	**Decision**
Lymph node status positive	2.02	1.41-2.88	8.00E-05	1	0.0038	Significant
HER2 status: HER2 negative	1.5	1.18-1.93	0.0011	2	0.0077	Significant
No restriction to subtype	1.41	1.13-1.77	0.0026	3	0.0115	Significant
ER Status: ER negative	3.19	1.43-7.11	0.0027	4	0.0154	Significant
PAM50 subtype: Her2	1.63	0.92-2.90	0.0907	5	0.0192	Insignificant
ER Status: ER positive	1.14	0.88-1.47	0.3184	6	0.0231	Insignificant
PGR status: PGR positive	1.11	0.84-1.46	0.4684	7	0.0269	Insignificant
PGR status: PGR negative	1.2	0.7-2.03	0.5086	8	0.0308	Insignificant
Lymph node status negative	1.11	0.81-1.51	0.5271	9	0.0346	Insignificant
PAM50 subtype: Basal	1.16	0.71-1.88	0.5535	10	0.0385	Insignificant
PAM50 subtype: Luminal B	1.07	0.68-1.68	0.7823	11	0.0423	Insignificant
HER2 status: HER2 positive	1.04	0.58-1.85	0.8976	12	0.0462	Insignificant
PAM50 subtype: Luminal A	0.98	0.67-1.43	0.913	13	0.0500	Insignificant

Reference:

1. Arnold, M.; Morgan, E.; Rumgay, H.; Mafra, A.; Singh, D.; Laversanne, M.; Vignat, J.; Gralow, J.R.; Cardoso, F.; Siesling, S.; et al. (2022). Current and future burden of breast cancer: Global statistics for 2020 and 2040. *Breast*, 66, 15–23
2. Baguley, T. 2012. *Serious stats: A guide to advanced statistics for the behavioral sciences*. Palgrave Macmillan 2012. [Online]. Available: https://books.google.fr/books?hl=fr&lr=&id=ObUcBQAAQBAJ&oi=fnd&pg=PP1& dq=baguley+2012 &ots=-eiUlHiCYs&sig= YUUKZ7jiGF33wdo3 WVO-8l-OUu8.
3. Blaes, A. H., Nair, C., Everson-Rose, S., Jewett, P., Wolf, J., & Zordoky, B., 2023. Psychological measures of stress and biomarkers of inflammation, aging, and endothelial dysfunction in breast cancer survivors on aromatase inhibitors. *Scientific Reports*, *13*(1). https://doi.org/10.1038/s41598-023-28895-4
4. Compas, B. E., & Luecken, L., 2002. Psychological Adjustment to Breast Cancer. Current Directions in Psychological Science, 11(3), 111-114. https://doi.org/10.1111/1467-8721.00180
5. Cowin, P., Rowlands, T. M., & Hatsell, S. J., 2005. Cadherins and Catenins in breast cancer. *Current Opinion in Cell Biology*, *17*(5), 499–508. https://doi.org/10.1016/j.ceb.2005.08.014
6. Delacre, M., Lakens, D., & Leys, C., 2017. Why psychologists should by default use Welch's T-test instead of student's T-test. *International Review of Social Psychology*, *30*(1), 92–101. https://doi.org/10.5334/irsp.82
7. Derrick, B., & White, P., 2016. Why Welch's test is type I error robust. *The Quantitative Methods for Psychology*, *12*(1), 30–38. https://doi.org/10.20982/tqmp.12.1.p030
8. Emmert-Streib, F., & Dehmer, M., 2019. Introduction to survival analysis in practice. *Machine Learning and Knowledge Extraction*, *1*(3), 1013–1038. https://doi.org/10.3390/make1030058
9. Erceg-Hurn, D., & Mirosevich, V., 2008. Modern robust statistical methods: An easy way to maximize the accuracy and power of your research. *American Psychologist,* 63(7). 591-601, doi: 10.1037/0003-066x.63.7.591
10. Etikan, I., 2017. The Kaplan Meier estimate in survival analysis. *Biometrics & Biostatistics International Journal*, *5*(2). https://doi.org/10.15406/bbij.2017.05.00128
11. Evangelou, E., & Ioannidis, J. P., 2013. Meta-analysis methods for genome-wide association studies and beyond. *Nature Reviews Genetics*, *14*(6), 379–389 https://doi.org/10.1038/nrg3472
12. Fekete, J. T., & Győrffy, B., 2019. ROCplot.org: Validating predictive biomarkers of chemotherapy/hormonal therapy/anti-her2 therapy using transcriptomic data of 3,104 breast cancer patients. *International Journal of Cancer*, *145*(11), 3140–3151. https://doi.org/10.1002/ijc.32369
13. Fekete, J. T., & Győrffy, B., 2019a. ROCplot.org: Validating predictive biomarkers of chemotherapy/hormonal therapy/anti-her2 therapy using transcriptomic data of 3,104 breast cancer patients. *International Journal of Cancer*, *145*(11), 3140–3151. https://doi.org/10.1002/ijc.32369
14. Fortin, J., Leblanc, M., Elgbeili, G., Cordova, M. J., Marin, M. F., & Brunet, A., 2021. The mental health impacts of receiving a breast cancer diagnosis: A meta-analysis. *British journal of cancer*, *125*(11), 1582–1592. https://doi.org/10.1038/s41416-021-01542-3
15. Iqbal, M. S., Ahmad, N., Mirza, Z., & Karim, S., 2023. Gene expression and survival analysis study of KIAA0101 gene revealed its prognostic and diagnostic importance in breast cancer. *Vegetos*, *36*(1), 249–258. https://doi.org/10.1007/s42535-022-00561-6

16. Jung, Y. Y., Kim, H. M., & Koo, J. S., 2015. Expression of lipid metabolism-related proteins in metastatic breast cancer. *PLOS ONE*, *10*(9). https://doi.org/10.1371/journal.pone.0137204
17. Karim S*, Merdad A, Schulten HJ, Jaypal M, Dallol A, Buhmeida A, Al-Thubaity F, Mirza Z, Gari MA, Chaudhary AGA, Abuzenadah AM, Al-Qahtani MH. Low expression of leptin and its associated with breast cancer: A transcriptomics study. Oncology Reports, May 2016, 36(1): 43-48.
18. Karim, S., Iqbal, M. S., Ahmad, N., Ansari, M. S., Mirza, Z., Merdad, A., Jastaniah, S. D., & Kumar, S., 2023. Gene expression study of breast cancer using Welch Satterthwaite T-test, Kaplan-Meier estimator plot and Huber loss robust regression model. *Journal of King Saud University - Science*, *35*(1), 102447. https://doi.org/10.1016/j.jksus.2022.102447
19. Karim, S.; Al-Kharraz, M.; Mirza, Z.; Noureldin, H.; Abusamara, H.; Alganmi, N.; Merdad, A.; Jastaniah, S.; Kumar, S.; Rasool, M.; et al., 2022 Development of "Biosearch System" for biobank management and storage of disease associated genetic information. *J. King Saud Univ.—Sci. 34*, 101760
20. Kim, S., Lee, Y., & Koo, J. S., 2015. Differential expression of lipid metabolism-related proteins in different breast cancer subtypes. *PLOS ONE*, *10*(3). https://doi.org/10.1371/journal.pone.0119473
21. Lánczky, A., & Győrffy, B., 2021. Web-based survival analysis tool tailored for Medical Research (kmplot): Development and implementation. *Journal of Medical Internet Research*, *23*(7). https://doi.org/10.2196/27633
22. Leung, K.-M., Elashoff, R. M., & Afifi, A. A., 1997. Censoring issues in survival analysis. *Annual Review of Public Health*, *18*(1), 83–104 https://doi.org/10.1146/annurev.publhealth.18.1.83
23. Martino, M.L., Lemmo, D., & Gargiulo, A., 2021. A review of psychological impact of breast cancer in women below 50 years old, Health Care for Women International, 42:7-9, 1066-1085, DOI: 10.1080/07399332.2021.1901901
24. Merdad A, Karim S*, Schulten H, Dallol A, Buhmeida A, Al-Thubaity F, Gari MA, Chaudhary AGA, Abuzenadah A, Al-Qahtani MH. Expression of Matrix Metalloproteinases (MMPs) in Primary Human Breast Cancer: MMP-9 as a Potential Biomarker for Cancer Invasion and Metastasis. Anticancer Research, Mar 2014, 34(3): 1355-1366.
25. Merdad A, Karim S*, Schulten H, Jaypal M, Dallol A, Buhmeida A, Al-Thubaity F, Gari MA, Chaudhary AGA, Abuzenadah A, Al-Qahtani MH. Transcriptomics profiling study of breast cancer from kingdom of Saudi Arabia revealed altered expression of adiponectin and fatty acid binding protein 4: Is lipid metabolism associated with breast cancer? BMC Genomics, Jan 2015, 16 (suppl 1): S11.
26. Mirza, Z., Ansari, M. S., Iqbal, M. S., Ahmad, N., Alganmi, N., Banjar, H., Al-Qahtani, M. H., & Karim, S., 2023. Identification of novel diagnostic and prognostic gene signature biomarkers for breast cancer using artificial intelligence and Machine Learning assisted transcriptomics analysis. *Cancers*, *15*(12), 3237. https://doi.org/10.3390/cancers15123237
27. Ramaswamy, S.; Tamayo, P.; Rifkin, R.; Mukherjee, S.; Yeang, C.H.; Angelo, M.; Ladd, C.; Reich, M.; Latulippe, E.; Mesirov, J.P.; et al.(2001) Multiclass cancer diagnosis using tumor gene expression signatures. *Proc. Natl. Acad. Sci. USA*, *98*, 15149–15154
28. Ruxton, G. D., 2006. The unequal variance T-test is an underused alternative to student's t-test and the mann–whitney U test. *Behavioral Ecology*, *17*(4), 688–690 https://doi.org/10.1093/beheco/ark016

29. Straub, B. K., Witzel, H. R., Pawella, L. M., Renner, M., Eiteneuer, E., Hashani, M., Schirmacher, P., Roth, W., & Mechtersheimer, G., 2019. Perilipin 1 expression differentiates liposarcoma from other types of soft tissue sarcoma. *The American Journal of Pathology*, *189*(8), 1547–1558. https://doi.org/10.1016/j.ajpath.2019.04.017
30. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F., 2021 Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.*, *71*, 209–249
31. Survival analysis: A practical approach, 2nd edition edited by Machin, D., Cheung, Y. B., and Parmar, M. K., 2007. *Biometrics*, *63*(1), 306–307 https://doi.org/10.1111/j.1541-0420.2007.00743_9.x
32. Sztalryd, C., & Kimmel, A. R., 2014. Perilipins: Lipid droplet coat proteins adapted for tissue-specific energy storage and utilization, and lipid cytoprotection. *Biochimie*, *96*, 96–101. https://doi.org/10.1016/j.biochi.2013.08.026
33. Tseng, G. C., Ghosh, D., & Feingold, E., 2012. Comprehensive literature review and statistical considerations for microarray meta-analysis. *Nucleic Acids Research*, *40*(9), 3785–3799. https://doi.org/10.1093/nar/gkr1265
34. Whitlock, M. C., 2005. Combining probability from independent tests: The weighted Z-method is superior to Fisher's approach. *Journal of Evolutionary Biology*, *18*(5), 1368–1373. https://doi.org/10.1111/j.1420-9101.2005.00917.x
35. Yang, H., Brand, J.S., Fang, F., Chiesa, F., Johansson, A.L.V., Hall, P. and Czene, K., 2017, Time-dependent risk of depression, anxiety, and stress-related disorders in patients with invasive and *in situ* breast cancer. Int. J. Cancer, 140: 841-852. https://doi.org/10.1002/ijc.30514
36. Yoon S, Baik B, Park T, Nam D., 2021 Powerful P-value combination methods to detect incomplete association. *Sci Rep*. https://doi.org/ 10.1038/s41598-021-86465
37. Zhang, X., Su, L., & Sun, K., 2021, May 15. *Expression status and prognostic value of the perilipin family of genes in breast cancer*. American journal of translational research. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC8205812
38. Zhou, C., Wang, M., Zhou, L., Zhang, Y., Liu, W., Qin, W., He, R., Lu, Y., Wang, Y., Chen, X.-Z., & Tang, J., 2016. Prognostic significance of PLIN1 expression in human breast cancer. *Oncotarget*, *7*(34), 54488–54502. https://doi.org/10.18632/oncotarget.10239

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