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ORIGINAL ARTICLE

Discovery of Lipid Metabolism Networks as Key Pathways in Breast Cancer via Genomic Data Integration and WGCNA

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SUMMARY

Background: **Breast cancer remains a major global health issue, requiring innovative approaches for early detection and treatment. This study employs weighted gene co-expression network analysis (WGCNA) to uncover the complex biological processes and pathways involved in tumorigenesis by focusing on gene modules rather than individual genes. The aim of this study was to integrate multiple datasets and utilize WGCNA to identify the key genes involved in breast cancer. By combining various gene expression datasets, we aimed to identify significant gene modules and regulatory networks that contribute to breast cancer progression**.

Methods: **Four gene expression datasets from the NCBI Gene Expression Omnibus (GEO) were integrated to explore the genetic profiles of breast cancer. Using high-throughput genomic data, WGCNA identified key regulatory networks and hub genes involved in disease progression, and RT-qPCR was performed for validation.**

Results: **The study identified 9,707 DEGs, showing significant alterations in gene expression between tumor and adjacent normal tissues. Four critical genes, ADIPOQ, CHRDL1, FABP4, and PLIN1, were highlighted, with their expression closely linked to lipid metabolism pathways, which are crucial in breast cancer biology. Notably, ADIPOQ expression was significantly reduced in tumor samples.**

Conclusions: **The integration of Omics data through WGCNA uncovered key interconnected gene modules, emphasizing the critical role of lipid metabolism in cancer progression. These results underscore the need for targeted therapeutic strategies to restore hub gene expression and to present potential biomarkers for early diagnosis**

and treatment. Moreover, lipid metabolism emerged as a pivotal pathway in breast cancer progression, suggesting that its regulation could be essential not only for targeted therapies but also for the prevention and control of the disease. This approach offers promising avenues for early intervention that could potentially reduce cancer risk.
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KEYWORDS

breast cancer, WGCNA, transcriptome analysis, ADIPOQ

INTRODUCTION

Breast cancer, a complex and multifaceted disease, continues to be a significant global health concern [1]. It is the most prevalent cancer among women, impacting millions of lives and representing a major cause of morbidity and mortality worldwide [2]. The intricate nature of breast cancer necessitates comprehensive exploration and innovative approaches for early detection, prognosis, and treatment [3].

In recent years, the advent of genomics has revolutionized our ability to unravel the molecular intricacies of breast cancer [4,5]. Genomic data analysis, particularly through high-throughput technologies, has become a cornerstone in cancer research, offering unprecedented insights into the genetic alterations, signaling pathways, and potential therapeutic targets underlying breast cancer [6,7]. This genomic perspective not only enhances our understanding of the disease's heterogeneity but also provides a foundation for personalized and targeted therapeutic interventions [8]. Therefore, gaining insights into the intricacies of breast cancer is imperative for identifying molecular biomarkers crucial for early diagnosis [9,10]. Despite advances in treatment and early detection, it remains one of the leading causes of cancer-related deaths among women globally [11,12]. Breast cancer is a highly heterogeneous disease characterized by diverse genetic and molecular profiles [13]. Genomic data analysis has emerged as a powerful tool in deciphering the genetic landscape of breast cancer. By delving into the complexities of gene expression patterns, mutational profiles, and regulatory networks, researchers can identify key molecular players involved in breast cancer initiation, progression, and response to treatment [14]. The emergence of high-throughput platforms for gene expression analysis is increasingly prominent [15]. Next-generation sequencing and microarray analyses have become foundational techniques in medical oncology, boasting diverse clinical applications such as molecular cancer classification, predicting tumor responses, prognostication, molecular diagnostics, the identification of new drug targets, and patient stratification [16]. Gene expression analysis has been instrumental in identifying differentially expressed genes (DEGs) that contribute to cancer development and progression. However, individual gene analysis often falls short of capturing the dynamic interactions and regulatory mechanisms within the cellular environment. To overcome these limitations, systems biology approaches, such as weighted gene co-expression network analysis (WGCNA), have been developed. One of the advanced methodologies used to analyze integrated data is WGCNA. WGCNA is a systems biology approach used to construct co-expression networks based on gene expression data, identifying modules of highly correlated genes. These modules can then be correlated with clinical traits or other phenotypic data to identify key regulatory networks and hub genes or the mRNAs that play pivotal roles in disease processes [17]. By integrating multiple genomic datasets, WGCNA can simultaneously analyze all available research on breast cancer, providing a more comprehensive view of its molecular mechanisms. This approach enhances the reliability of findings by identifying critical pathways and hubs with greater accuracy, offering valuable insights into disease progression and potential therapeutic strategies. Integrating genomic data offers a holistic view of the alterations driving breast cancer [17,18]. By combining data from gene expression profiles and mRNA sequencing datasets, researchers can identify differentially expressed mRNAs in breast cancer [19]. The integration of data, including genomics, offers a holistic view of the genomic alterations that drive breast cancer, paving the way for more precise diagnostics and therapeutic strategies.

WGCNA is particularly valuable in cancer research as it helps uncover the underlying biological processes and pathways associated with tumorigenesis [20]. By focusing on gene modules rather than individual genes, WGCNA provides insights into the coordinated regulation of gene expression, revealing complex interactions and dependencies that may not be apparent through tradi-

tional differential expression analysis. This network-based approach facilitates the identification of central regulators within the network, which are often crucial for maintaining the malignant phenotype of cancer cells. The comprehensive analysis of mRNA interactions through integrated datasets not only enhances our understanding of breast cancer but also holds promise for improving patient outcomes through more targeted and personalized treatment approaches [21].

This study aimed to leverage the wealth of data available through gene expression profiling to gain a comprehensive understanding of the molecular landscape of breast cancer. Therefore, we integrated datasets GSE-45827, GSE65194, and GSE185645 to identify the key genes. We also performed WGCNA on the combined datasets to uncover crucial pathways involved in breast cancer. Furthermore, the identification of gene modules, or clusters of co-expressed genes, can provide insights into the biological processes and pathways associated with breast cancer. This approach will enhance our understanding of the significant genes related to this disease.

MATERIALS AND METHODS

Data preprocessing

Raw data from four datasets containing breast cancer and control samples was downloaded from the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo). This study integrated four GSEs to comprehensively analyze differential gene expressions (DEG) and construct WGCNA. The selected datasets GSE45827, GSE65194, GSE185645, and GSE42568 collectively include 442 samples, consisting of 402 disease samples and 40 normal samples. The samples encompass a diverse set of diseases, with GSE45827 comprising 130 diseases and 11 normal samples, GSE65194 comprising 153 diseases and 11 normal samples, GSE1-85645 comprising 15 diseases and 1 normal sample, and GSE42568 comprising 104 diseases and 17 normal samples. Three out of the four datasets shared identical platforms, and all four datasets were Affymetrix-based, thereby minimizing batch effects. All datasets were processed using the 'affy' package for reading and normalization, except for GSE185645, which was handled using the 'oligo' package. Robust multi-array average (RMA) normalization was performed using the 'rma' function for all datasets. The integrated expression matrix was then subjected to the ComBat function from the 'sva' package to mitigate potential batch effects and enhance the reliability of downstream analyses. This resulted in a total of 402 diseases and 40 normal samples for analysis. Differential expression analysis identified genes with significant expression changes between disease and normal samples. Additionally, WGCNA was employed to construct co-expression networks and identify gene modules associated with specific traits [22].

Microarray data and identification of DEGs

Integrated datasets were merged to create a comprehensive dataset, and the ComBat function was applied. Gene filtering was then performed using the genefilter package. Out of 16,554 genes, 9,707 genes were selected as DEGs [23]. The resulting DEGs were those with adjusted p-value < 0.001, p-value < 0.001, and |logFC| > 2. This methodology aims to identify robust DEGs by combining gene filtering and differential expression analysis techniques. The stringent criteria applied during the limma analysis ensured the selection of genes with high statistical significance and substantial fold changes. The information for the samples analyzed in this study is listed in Table 1.

Table 1. DEGs analysis of selected datasets.

Dataset	Type	Sample		Total
		Normal	Breast cancer	442
GSE45827	mRNA	11	130	141
GSE65194	mRNA	11	153	164
GSE185645	mRNA	1	15	16
GSE42568	mRNA	17	104	121

Construction of gene co-expression network and identification of significant modules

To perform WGCNA on the provided datasets, a systematic methodology was adopted [24]. The WGCNA approach was employed to reconstruct the co-expression network of breast cancer genes. To outline the procedure, the gene expression matrices underwent a transformation into matrices representing the similarities of paired mRNAs. Subsequently, these matrices were converted into adjacency matrices utilizing the Pearson correlation coefficient test. The process involved determining the smallest feasible β-value by applying the scale-free topology technique for gene co-expression to ensure that the resulting adjacency matrix aligned with the required scale-free topology criteria.

To filter genes for WGCNA, we applied a criterion of coefficient of variation (CV) greater than 0.1. Genes exhibiting a CV exceeding 0.1 were considered to have the most substantial changes and were selected for further WGCNA analysis. Subsequently, a total of 4,122 genes were subjected to WGCNA analysis based on their CV values. Following gene filtration, clustering of samples was performed. An outlier, identified as sample GSM1045248, was excluded from the analysis. As a result, 441 samples were retained for subsequent stages of the WGCNA analysis. The soft threshold value was set to 10, equivalent to 2 raised to the power of 0.9 in R. This step was crucial for constructing the adjacency matrix and forming co-expression modules in the subsequent stages of the analysis. With the soft threshold established, the next steps involved creating the adjacency

matrix and forming modules. These modules represented groups of genes with similar co-expression patterns, providing a basis for understanding the underlying regulatory networks in the dataset. By employing this comprehensive methodology, the WGCNA analysis aimed to identify key genes, explore co-expression patterns, and unravel the potential biological significance of the identified modules in the context of the studied biological system, and integrated analysis and functional enrichment analysis were analyzed by EnrichR.

Patients and demographics

Breast tissues from 25 patients diagnosed with breast cancer, including 25 tumor samples and 25 adjacent normal tissues, were obtained from the Seyed Shahada Hospital between June 2017 and November 2018. The inclusion criteria required confirmation through pathological and genetic assessments, and patients with coexisting conditions such as autoimmune diseases, diabetes, cardiovascular conditions, or a history of chemotherapy or radiotherapy were excluded from the sampling cohort. The study strictly adhered to ethical principles as outlined in the Helsinki Declaration, obtaining approval from the ethics committee at branch shahrekord University of Medical Sciences (IR.SHK.REC. 1401.113). Demographic characteristics of breast cancer patients are shown in Table 2.

Validation of the expression of selected genes by RT-qPCR

Total mRNA from tissue samples was extracted using TRIzol (Invitrogen; Thermo Fisher Research, Inc.) following the manufacturer's standard protocol. Following quantification and qualification of RNA samples using a NanoDrop spectrophotometer (Epoch spectrophotometer-BioTek) and standard agarose gel electrophoresis, respectively, RNA was reverse transcribed into cDNA using the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, USA) according to the manufacturer's instructions. Subsequently, RT-qPCR was conducted using RealQ Plus Master Mix Green (Amplicon) and the Applied Biosystems 7500 real-time PCR system (Thermo Fisher Scientific, Inc., Waltham, MA, USA). The primer sequences utilized in RT-qPCR are provided in Table S1. In this process, GAPDH served as an endogenous control for normalizing expression levels, ensuring standardized relative expression levels for subsequent data analysis. The data were then analyzed using the 2-ΔΔCT method, with each sample examined in triplicate [25,26].

Statistical analysis

RT-qPCR results were analyzed using SPSS (version 16.0; SPSS Inc, Chicago, IL, USA), and GraphPad Prism 6.0 (GraphPad Software, San Diego, CA, USA) software. The Shapiro-Wilk normality test was used to measure the normality of the variables among two groups. The statistical significance of the discrepancy between normally distributed variables was calculated via unpaired Student's *t*-test. A p-value of less than 0.05 was considered statistically significant [27].

Table 2. Demographic characteristics of breast cancer patients.

Characteristic	Number of patients (n)	Percentage (%)
Total patients	**25**	**100**
Age (years)		
< 40	**4**	**16**
40 - 49	**7**	**28**
50 - 59	**6**	**24**
≥ 60	**8**	**32**
Gender		
Female	**25**	**100**
Male	**0**	**0**
Menopausal status		
Pre-menopausal	**11**	**44**
Post-menopausal	**14**	**56**
Family history of BC		
Yes	**10**	**40**
No	**15**	**60**
Tumor stage		
Stage I	**6**	**24**
Stage II	**11**	**44**
Stage III	**5**	**20**
Stage IV	**3**	**12**
Histological type		
Ductal carcinoma	**17**	**68**
Lobular carcinoma	**4**	**16**
Other	**4**	**16**
Receptor status		
ER+	**16**	**68**
PR+	**14**	**56**
HER2+	**8**	**32**
Triple-negative	**3**	**12**

RESULTS

DEG identification

Four expression datasets containing breast cancer and control samples were used to investigate the role of mRNAs in breast cancer. After merging the data, the ComBat command from the sva package was used to reduce batch effects (Figure 1). Quantile normalization was performed to minimize technical noise in each dataset. In the next step, DEG analysis was carried out. Genes with an adj-p < 0.001, p-value < 0.001, and |logFC| > 2 were filtered and selected, resulting in 2

Table 3. Characteristics of selected modules.

Module	Correlation	p-value	Genes	Real hub genes (wgcna + DEG)
First	**-0.86**	**6e - 128**	**72**	**ADIPOQ, CHRDL1, FABP4, PLIN1**
Second	**-0.44**	**1e - 22**	**45**	
Third	**0.42**	**9e - 21**	**95**	

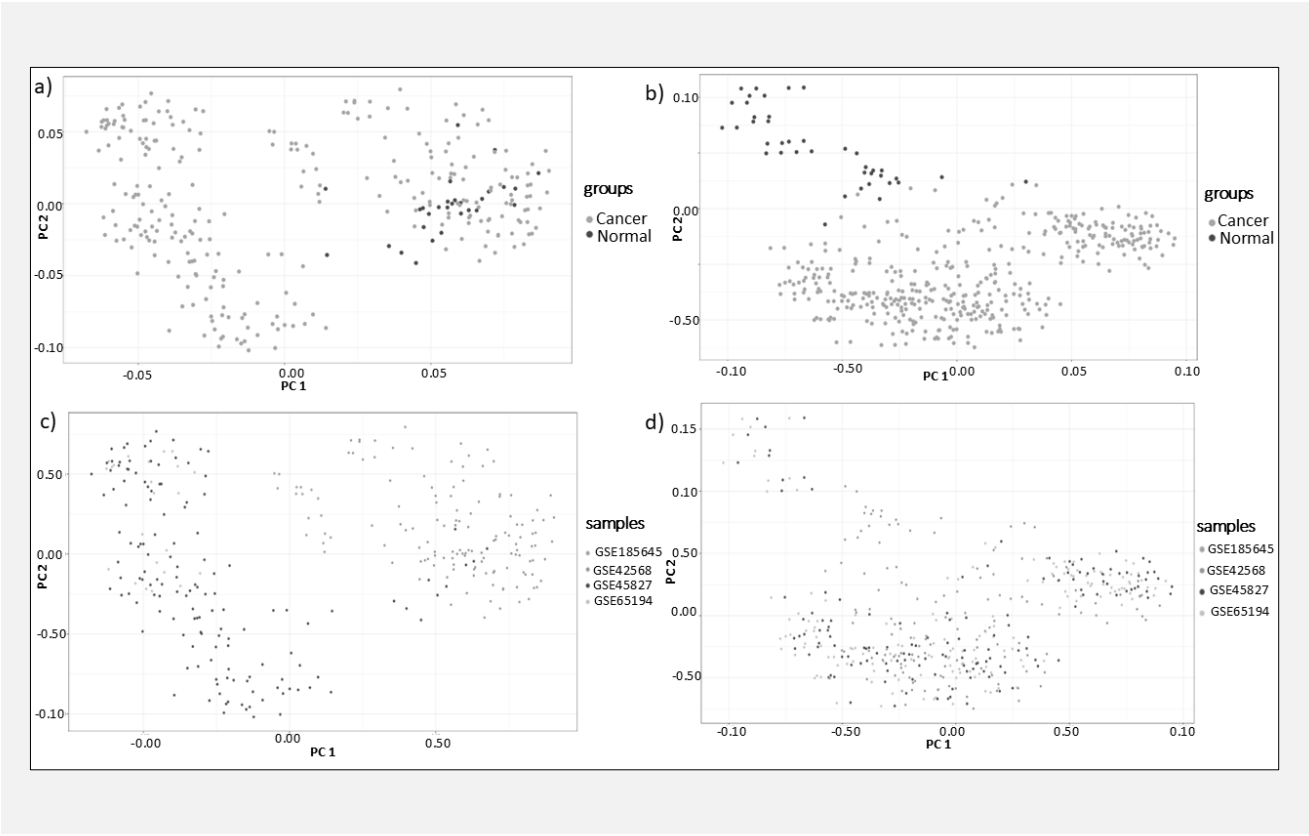

Figure 1. ComBat results and reduction of the batch effect.

a) Data dispersion before ComBat based on groups, b) the reduction of the batch effect of the data and the reduction of the batch effect of the groups, c) data dispersion before ComBat based on GSEs, and d) the reduction of the batch effect of the data and the reduction of the batch effect of the GSEs.

overexpressed genes and 7 downregulated genes (Table 3). A heatmap of gene co-expression was created to check correlations (Figure 2a). Volcano plots of DEGs results are displayed in Figure 2b, and DEG analysis was performed to find the most differentiated expressed genes (Figure 3).

Identification of clinically significant modules

A total of 4,122 genes with a coefficient of variation (CV) greater than 0.1 were regarded as having the largest changes and were selected for WGCNA analysis. A soft threshold (β) was selected with a cutoff R^2 value of 0.9 (Figure S1b). With this β value, the networks closely resemble the real biological network state as they adhere to the power law distribution. Following that, a hierarchical clustering analysis based on weighted correlation was done, and the clustering results were segmented based on the established criteria for obtaining gene modules. The first module had the highest correlation with 72 gene hubs, the second module with 45 hubs, and the third module with 95 gene hubs. All gene co-expression modules were visualized in Figure 4 and Table 3. WGCNA cluster dendrogram and module assignment. The branches refer to clusters of genes that

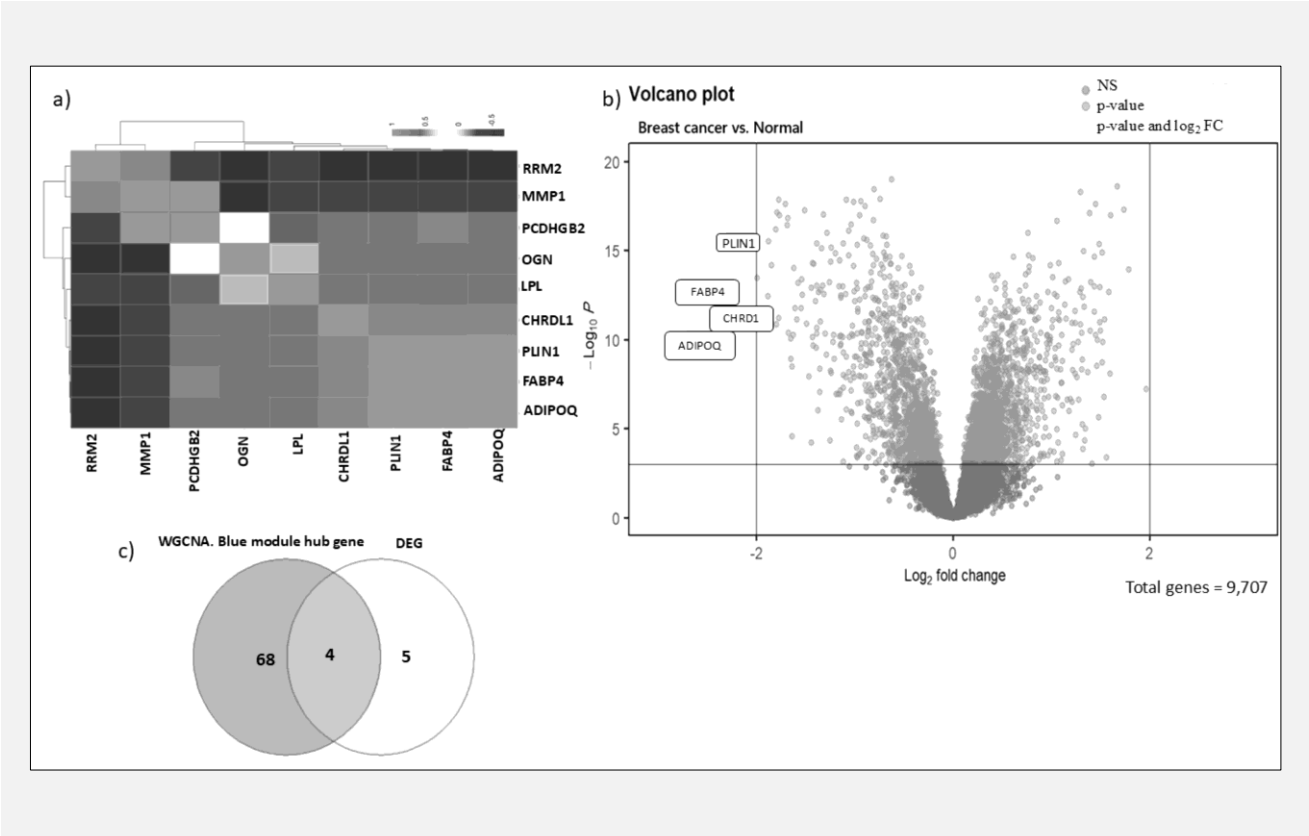

Figure 2. a) The co-expression heat graph of genes was drawn to check the correlation. b) Volcano plots of DEGs. The vertical axis is the mean value of -log 10 (false discovery rate) and the horizontal axis is the value of LogFC. Red dots present the significant dysregulated genes that meet the criteria. c) Venn diagram of DEGs and modules real hub-gene selection.
The overall analysis of this study was performed in two separate manners. In the first one, DEG analysis was performed to find the most differentiated expressed genes (List 1). In Parallel, WGCNA was done to find genes with the most values of 'gene significance' and 'module membership', which present the weight of genes in the network.

are highly connected (Figure S1b). We selected modules with the highest negative correlation for enrichment analysis, and the genes in the first module were analyzed using the functional enrichment tool EnrichR (Figure 5).

Identification of real hub and Venn diagram

In the integrated analysis, the Venn diagram revealed the shared genes between the modules identified by WGCNA and the genes resulting from differential expression analysis (DEG), emphasizing the interconnected molecular landscape between WGCNA hub-genes and DEG-derived genes. The Venn diagram was created using R software.

Analysis of the expression status of selected genes by RT-qPCR

To validate in silico analysis results, RT-qPCR was performed on cDNA from breast cancer and control tissue samples. As results are shown in Figure 6, the expression of ADIPOQ in tumor breast cancer samples had significantly decreased relative to the control. Furthermore, elevated expression of CHRDL1 was observed in Figure 6a, b. Additionally, we found that FABP4 was downregulated in the tumor breast cancer samples (Figure 6c), and increased expression of PLIN1 was observed in Figure 6d. Additionally the biomarker analysis of ADIPOQ, CHRDL1, FABP4, and PLIN1 indicated that all genes except PLIN1 exhibit biomarker potential (Figure 6).

DISCUSSION

WGCNA is a critical tool for integrating diverse datasets to uncover significant pathways in cancer research. Its ability to identify gene modules and hub genes associated with cancer phenotypes makes it valuable for discovering therapeutic targets. Genomic data integration and WGCNA are essential tools in identifying key genes and pathways in breast cancer, providing profound insights into the molecular mechanisms underlying disease progression. These methods allow for comprehensive analysis of diverse datasets, facilitating the

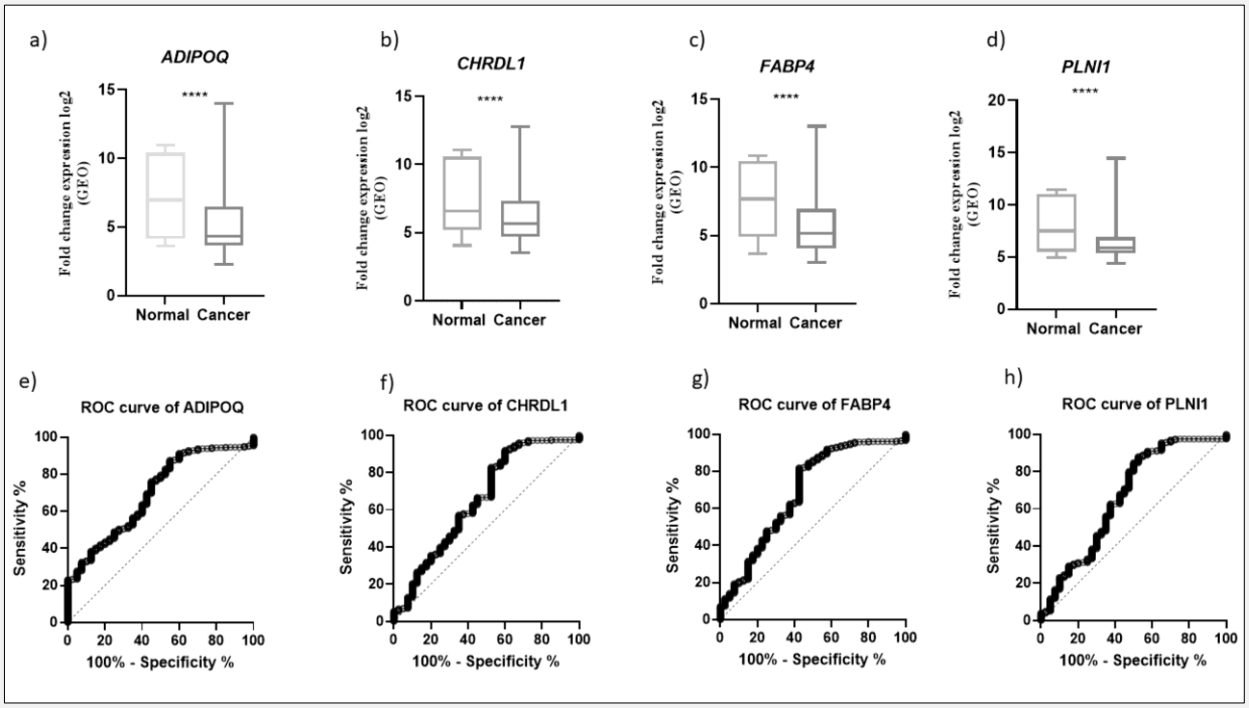

Figure 3. DEG analysis was performed to find the most differentiated expressed genes.

a) Expression of ADIPOQ in normal samples and tumor samples from GEO, p-value < 0/0001 FDlog2 = -1/799 ± 0/4,076. b) Expression of CHRDL1 in normal samples and tumor samples from GEO, p-value < 0/0001 FDlog2 = -1/405 ± 0/3,240. c) Expression of FABP4 in normal samples and tumor samples from GEO, p-value < 0/0001 FDlog2 = -1/783 ± 0/3,499. d) Expression of PLIN1 in normal samples and tumor samples from GEO, p-value < 0/0001 FDlog2 = -1/528 ± 0/2873. e) ROC analysis of ADIPOQ expression showing promising discrimination power between tumor and non-tumor tissues in breast area = 0/6,986, p-value < 0/0001. f) ROC analysis of CHRDL1 expression showing promising discrimination power between tumor and non-tumor tissues in breast area = 0/6,552, p-value < 0/0012. g) ROC analysis of FABP4 expression showing promising discrimination power between tumor and non-tumor tissues in breast area = 0/6,897, p-value < 0/0001. h) ROC analysis of PLIN1 expression showing promising discrimination power between tumor and non-tumor tissues in breast area = 0/6,673, p-value < 0/0005.
(List 2). The similar genes between the two lists were selected as real hub genes.

discovery of gene modules and advancing our understanding of biological pathways, which is crucial for developing personalized therapies. Despite these benefits, several challenges are associated with their application. WGCNA and genomic integration demand considerable computational resources, and the variation across studies can limit the consistency of results. Additionally, integrating datasets from different platforms poses challenges, as does the interpretation of gene modules without clear biological relevance. Other issues include dataset selection bias, overfitting, and the risk of false positives, which require rigorous validation and a cautious approach in cancer research.

Regardless of these challenges, the potential for these methods to improve cancer research and precision medicine remains significant. WGCNA has proven valuable in identifying gene co-expression networks across various cancer types - including lung, colon, prostate, and ovarian cancers - by pinpointing biomarkers, therapeutic targets, and molecular mechanisms associated with tumorigenesis and progression. Such applications underscore its role in advancing personalized medicine approaches [28]. Based on the results, it appears that lipid metabolism pathways play a central role in breast cancer progression. While previous studies have sporadically mentioned the involvement of metabolic genes in breast cancer, often highlighting their oncogenic potential, our comprehensive approach, which integrated multiple datasets and utilized WGCNA, has identified the lipid metabolism pathway as one of the key factors in this disease. The expression patterns of genes such as ADIPOQ, FABP4, and PLIN1, as well as their biomarker capabilities, further underscore the significance of lipid metabolism in breast cancer. These findings suggest that targeting lipid metabolic pathways could be crucial not only for understanding the disease's pro-

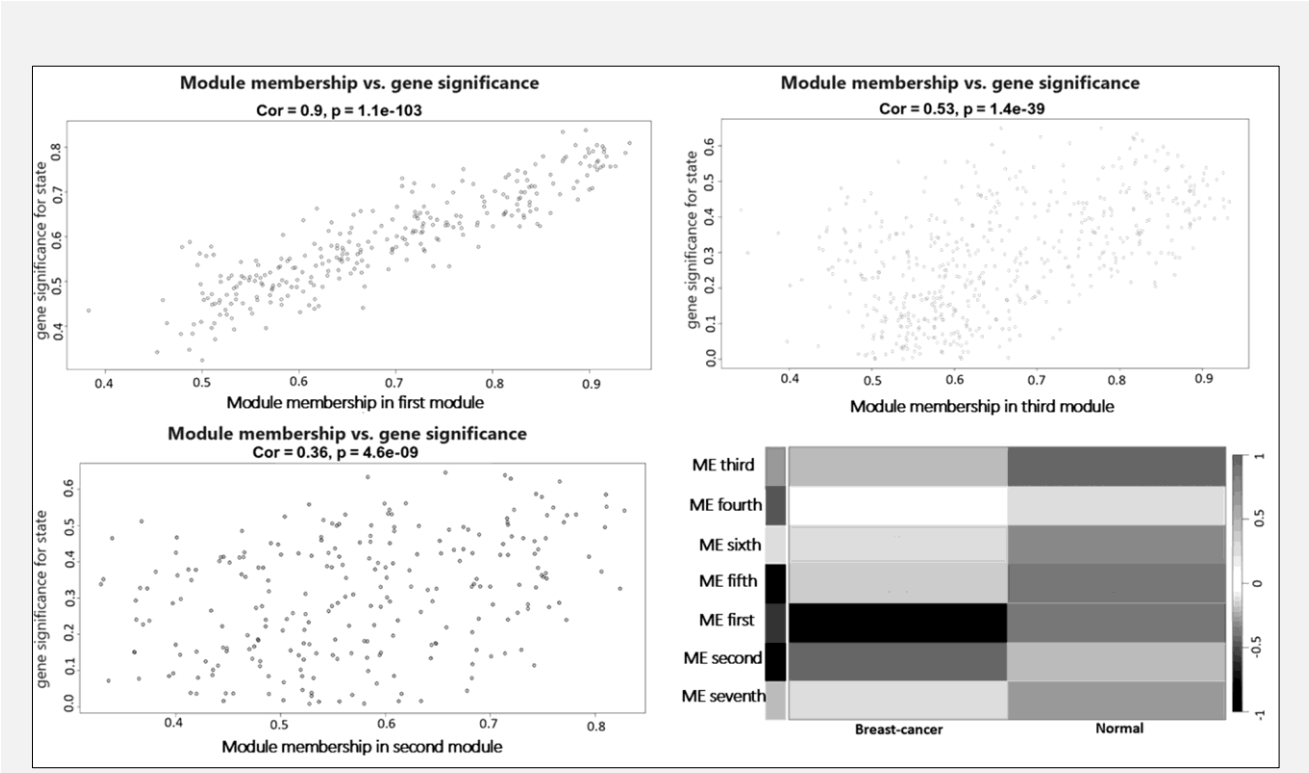

Figure 4. Module trait relationship and module features of GS and MM.

Every point defines a specific gene within every module that is plotted on the y-axis and the x-axis by GS and MM, respectively. a) first module (correlation: -0.86, p-value: 6e-128), b) second module (correlation: -0.44, p-value: 1e-22), c) third module (correlation: 0.42, p-value: 9e-21), and d) heat map and gene expression changes of the modules.

gression but also for developing more effective preventive and therapeutic strategies.

Adiponectin (ADIPOQ), a hormone secreted by adipocytes, plays a crucial role in regulating glucose and fatty acid metabolism. Its involvement in cancer has been widely studied due to its impact on various signaling pathways associated with tumorigenesis [29]. In this study, we showed that the expression of adiponectin in the tumor tissue was decreased compared to the adjacent non-tumor tissue. In previous studies, it was also shown that low levels of adiponectin increase the risk of breast cancer. Adiponectin exerts anticancer effects by activating the AMPK pathway, which maintains cellular energy balance, and inhibiting the mTOR pathway, which is essential for cell growth. This dual mechanism inhibits the proliferation of breast cancer cells by inducing cell cycle arrest and apoptosis. In colorectal cancer (CRC), lower adiponectin levels are linked to higher disease risk and progression [30]. Adiponectin inhibits the NF-κB signaling pathway, which is crucial in inflammation and cancer development. Additionally, it activates the AMPK pathway in CRC cells, reducing proliferation and promoting apoptosis. Studies have shown that higher adiponectin levels correlate with a lower risk of advanced prostate cancer [31,32]. Adiponectin inhibits prostate cancer cell proliferation by modulating insulin-like growth factor (IGF) and its downstream pathways, including PI3K/Akt and MAPK/ERK, critical for cell survival and proliferation [33-35]. Reduced expression of ADIPOQ diminishes its regulatory effects on crucial signaling pathways involved in cell growth and apoptosis. Low adiponectin levels lead to decreased activation of the AMPK pathway and less inhibition of the mTOR pathway, resulting in unchecked cellular proliferation and tumor growth. Furthermore, insufficient adiponectin fails to inhibit NF-κB signaling effectively, leading to enhanced inflammation and a pro-tumorigenic environment. This creates a favorable condition for cancer initiation and progression. In summary, the reduction in ADIPOQ expression disrupts the balance of key signaling pathways, promoting an environment conducive to cancer development and progression. This underscores the importance of maintaining adequate adiponectin levels as a potential strategy for cancer prevention and treatment. CHRDL1 (chordin-like 1) is a gene encoding a protein that regulates

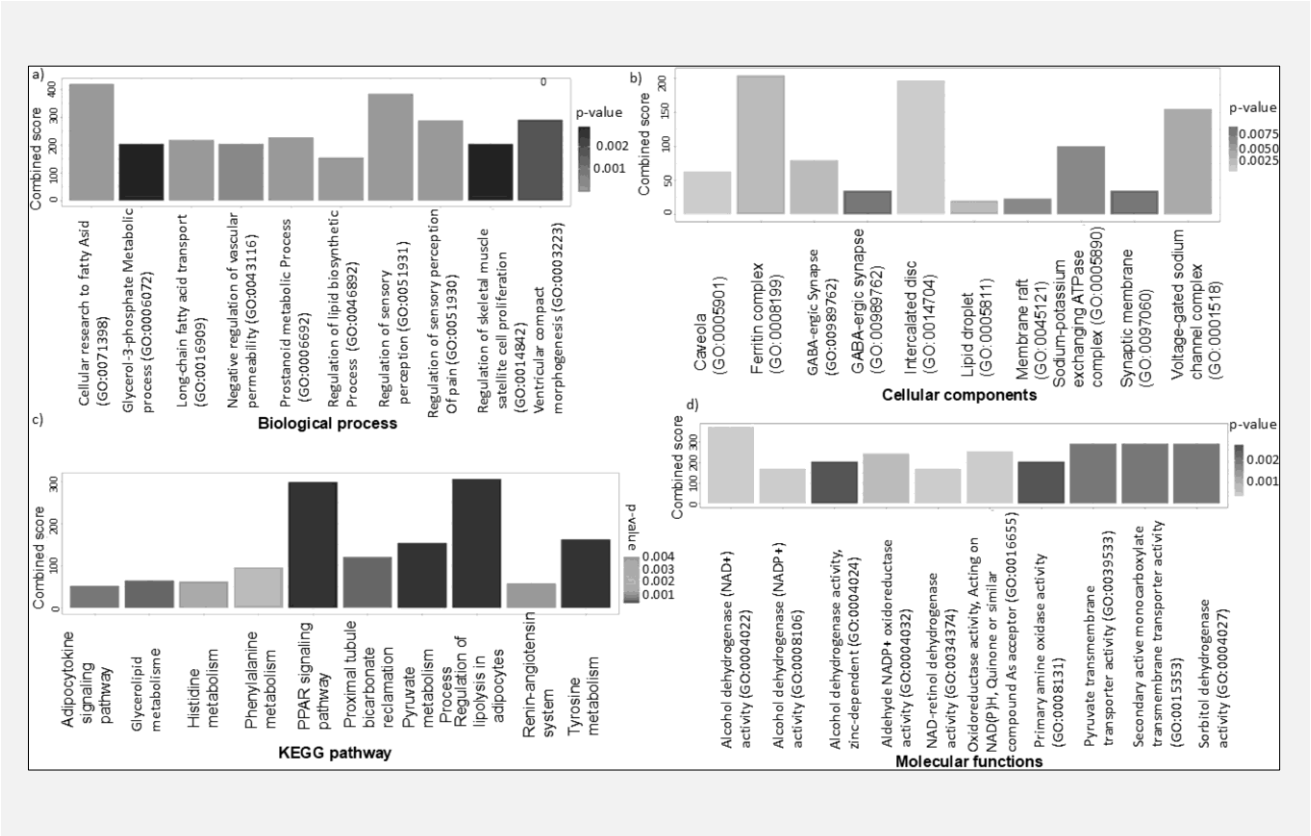

Figure 5. Enrichment analysis of first module.
a) The highest score for biological process enrichment results has cellular response to fatty acid, b) the highest score for cellular component enrichment results has lipid droplet, c) the highest score for keeg pathway enrichment results has regulation of lipolysis in adipocytes, and d) the highest score for molecular function enrichment results has alcohol dehydrogenase.

growth factors like bone morphogenetic proteins (BMPs), which are crucial for cell growth, differentiation, and apoptosis. Dysregulation of BMP signaling is implicated in cancer progression and metastasis. Decreased expression of CHRDL1, observed in breast cancer and other cancers, has contributed to uncontrolled cell proliferation and resistance to apoptosis, hallmark traits of cancer. CHRDL1 acts as a tumor suppressor by negatively regulating BMP signaling. It binds to BMPs, preventing their interaction with receptors, thereby inhibiting BMP activity. In breast cancer, reduced CHRDL1 leads to increased BMP activity, promoting tumor growth and metastasis by enhancing cell proliferation and inhibiting apoptosis. Epigenetic changes, such as DNA methylation and histone acetylation, are associated with decreased CHRDL1 expression in breast cancer [36]. These changes can silence the CHRDL1 gene, reducing its tumor-suppressive effects. Mechanistically, the loss of CHRDL1 results in overactivation of BMP signaling pathways, leading to increased cell proliferation. Additionally, reduced CHRDL1 expression decreases apoptosis, allowing cancer cells to survive longer and accumulate mutations. The loss of CHRDL1 also enhances the invasive potential of cancer cells through epithelial-mesenchymal transition (EMT), a process linked to metastasis. Clinically, the downregulation of CHRDL1 in breast cancer suggests its potential as a biomarker for early detection and prognosis. Restoring CHRDL1 function or targeting BMP signaling could provide new therapeutic strategies. Developing drugs that mimic CHRDL1 activity or inhibit BMP receptors may help control cancer progression and improve patient outcomes. Fatty acid binding protein 4 (FABP4) is a vital lipid chaperone that plays a vital role in the transport of fatty acids and lipophilic substances, as well as in lipid metabolism and energy homeostasis. Research has linked FABP4 to various cancers, including breast, prostate, and ovarian cancer, due to its role in metabolic regulation. Studies show that FABP4 is overexpressed in breast cancer tissues and is associated with tumor aggressiveness and poor prognosis. By modulating lipid metabolism, this substance promotes the proliferation and survival of cancer cells and facilitates the supply of energy to rapidly growing tumor cells [37]. Higher levels of FABP4 are associated with increased risk and progression of prostate cancer. It supports the proliferation of cancer cells by increasing lipid accumulation and energy metabolism. FABP4 also con-

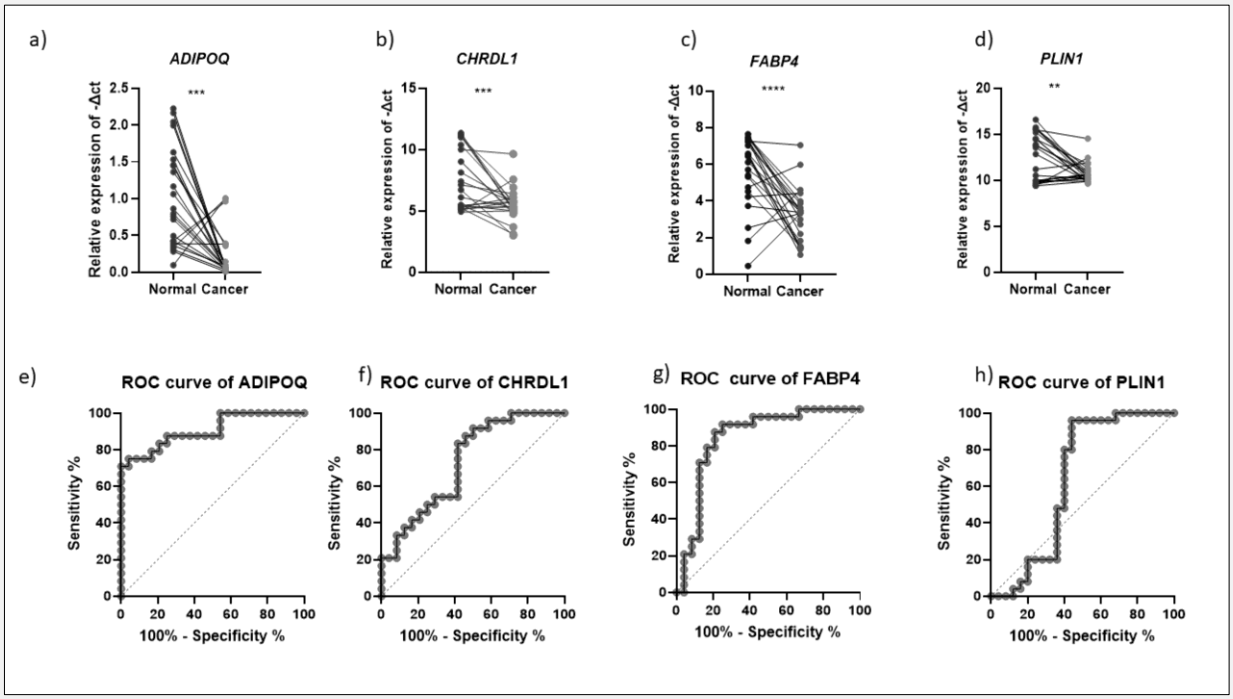

Figure 6. RT-qPCR experiments of ADIPOQ, CHRDL1, FABP4, and PLIN1.

a) Expression levels of ADIPOQ in paired tumor and adjacent samples of breast p-value = 0/0001 FDlog2 = -0/8,004, b) expression levels of CHRDL1 in paired tumor and adjacent samples of breast p-value < 0/0007 FDlog2 = -2/102 ± 0/5,764, c) expression levels of FABP4 in paired tumor and adjacent samples of breast p-value < 0/0001 FDlog2 = -2/445 ± 0/5,002, d) expression levels of PLIN1 in paired tumor and adjacent samples of breast p-value < 0/0013 FDlog2 = -1/961, e) ROC analysis of ADIPOQ expression showing promising discrimination power between tumor and non-tumor tissues in breast area = 0/9,045, p-value < 0/0001, f) ROC analysis of CHRDL1 expression showing promising discrimination power between tumor and non-tumor tissues in breast area = 0/7,309, p-value < 0/0012, g) ROC analysis of FABP4 expression showing promising discrimination power between tumor and non-tumor tissues in breast area = 0/8,455, p-value < 0/0061, and h) ROC analysis of PLIN1 expression showing promising discrimination power between tumor and non-tumor tissues in breast area = 0/6,384, p-value < 0/0933.

tributes to the progression of ovarian cancer by transferring fatty acids to cancer cells, their growth, and metastasis. FABP4 regulates lipid metabolism essential for energy production in cancer cells, supports inflammation and immune responses through NF-κB signaling, promotes angiogenesis, and promotes tumor growth and metastasis [38,39]. In some contexts, decreased expression of FABP4 in breast cancer can disrupt lipid metabolism and energy homeostasis and lead to metabolic reprogramming of cancer cells. This can increase the proliferation and survival of malignant cells, which is mediated by mechanisms such as DNA methylation and histone acetylation, which results in the silencing of the FABP4 gene. Perilipin 1 (PLIN1) is a key lipid droplet-associated protein that regulates lipid storage and metabolism within adipocytes. This gene has been increasingly studied in the context of cancer, given its role in managing lipid metabolism, which is often dysregulated in cancer cells. Research like ours shows that PLIN1 expression is downregulated in breast cancer tissues, disrupts lipid metabolism, and supports cancer cell growth. This reduction is associated with increased tumor aggressiveness and poor prognosis, as it promotes lipolysis, providing free fatty acids for rapid energy production and tumor proliferation. Although studies of PLIN1 in other cancers are limited, changes in lipid metabolism suggest that altered PLIN1 expression may also affect these cancers. PLIN1 is essential for regulating lipid droplet dynamics, and its loss causes the breakdown of stored lipids and tumor growth. In addition, PLIN1 depletion disrupts lipid homeostasis and activates signaling pathways such as PI3K/Akt, which promotes cell survival and resistance to apoptosis [40]. This reduction in PLIN1 expression can occur through genetic mutations or epigenetic changes, diminishing its tumor-suppressive effects. Understanding the role of PLIN1 highlights its potential as a biomarker for early cancer diagnosis and treatment. Targeting PLIN1 or lipid metabo-

lism pathways may provide new strategies to control tumor growth and improve patient outcomes. Overall, PLIN1 is very important in cancer biology, especially in breast cancer, emphasizing its therapeutic potential.

The main purpose of this study was to scrutinize the prognostic significance, functional role, and expression pattern of fatty acid metabolism genes in breast cancer. Among the identified genes, ADIPOQ plays a key role in lipid metabolism and has been shown to regulate fatty acid oxidation and glucose levels. In breast cancer, altered levels of adiponectin have been associated with tumor growth and progression, potentially influencing how cancer cells use lipids for energy. While primarily known for its role in developmental processes, CHRDL1 has been linked to lipid metabolism. Changes in its expression may affect cell signaling pathways that control lipid metabolism, contributing to breast cancer progression by affecting the tumor microenvironment. CHRDL1 is crucial for the uptake and transport of fatty acids within cells. In breast cancer, increased expression of FABP4 may enhance lipid uptake, supporting the energy needs of rapidly growing cancer cells and promoting their survival. PLIN1 is involved in lipid droplet formation and regulation of lipolysis. Its dysregulation in breast cancer could lead to abnormal lipid storage and release, potentially promoting cancer cell growth by providing an energy source through lipolysis. These genes are essential for regulating lipid processes, and their dysregulation in breast cancer can drive disease progression by influencing lipid metabolism and energy use in cancer cells.

These genes are essential for regulating lipid processes, and their altered expression in breast cancer may contribute to disease progression by affecting how cancer cells metabolize and utilize lipids. While each of these genes has previously been studied individually in breast cancer, our approach is unique because it provides a comprehensive understanding of their collective roles and interactions. This integration of data allows us to reveal new insights that would not be revealed from studying each gene in isolation. Besides, we used bioinformatics data integration to increase the robustness of our findings. In addition to examining each of these genes in breast cancer, the results of data integration and enrichment of identified genes showed that fatty acid-related pathways are among the most important pathways in which the key genes are involved. Previous research has particularly highlighted the role of each of these genes as influential factors in lipid metabolism. In this study, after enriching the genes associated with breast cancer, their significance in the lipid and fatty acid pathways was emphasized. This is due to the highest score in the biological process enrichment results being attributed to cellular responses to fatty acids. The highest scores in the cellular component enrichment and KEGG pathway analyses were related to lipid droplets and the regulation of lipolysis in adipocytes, respectively. Finally, the highest score for molecular function enrichment results was associated with alcohol dehydrogenase activity.

In conclusion, our study emphasizes the significant role of ADIPOQ, CHRDL1, FABP4, and PLIN1 in breast cancer, particularly in regulating lipid metabolism pathways. By using WGCNA and integrating GSE datasets, we have not only confirmed the individual importance of these genes, but also elucidated their collective interactions and contributions to tumor biology. Our findings suggest a potential link between lipid metabolism pathways and breast cancer progression, yet further studies are needed to fully clarify the precise mechanisms involved. Existing evidence shows that alterations in lipid profiles, such as changes in lipid composition and BMI, may influence tumor growth and treatment response. However, the specific role of lipid metabolism in cancer progression remains unclear and requires deeper investigation, particularly regarding the phenotypic characteristics of these alterations in breast cancer patients. Such insights could inform both targeted therapies and preventive strategies.

Our results highlight the importance of maintaining appropriate expression levels of key genes involved in lipid metabolism, positioning them as potential biomarkers for early detection and therapeutic targeting in breast cancer. This integrated approach underscores the value of further research to unravel cancer biology's complexities and develop innovative treatments. Additionally, identifying lipid metabolism as a critical pathway not only offers potential for clinical diagnosis and therapy but also opens avenues for preventive measures. By focusing on lifestyle changes, such as managing lipid intake and body fat, there is significant potential to reduce the risk of developing breast cancer. This dual strategy, addressing both prevention and treatment, holds promise for improving patient outcomes and promoting long-term health.

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The authors declare that they have no conflicts of interest related to this study.

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