RESEARCH ARTICLE

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Comprehensive Analysis of the Expression, Prognosis, and Immune Infiltrates for Chromodomain-Helicase-DNA-Binding Proteins in Breast Tumor

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Abstract

Background: Several recent studies suggest that chromodomain-helicase -DNA-binding domains (CHDs) are linked with cancers. We explored the association between chromodomain-Helicase-DNA-binding domain proteins and breast cancer (BrCa) and introduced potential prognostic markers using various databases. **Materials and Methods:** We analyzed the expression of the CHD family and their prognostic value in BrCa by mining UALCAN, TIMER, and Kaplan-Meier plotter databases. The association of CHD expression and immune infiltrating abundance was studied via the TIMER database. In addition, microRNAs related to the CHD family were identified by using the MirTarBase online database. **Results:** The present study indicated that compared to normal tissues, BrCa tissues showed increased mRNA levels of CHD3/4/7 but decreased CHD2/5/9 expression. Interestingly, We also found a positive correlation between CHD gene expression and the infiltration of macrophage, neutrophil, and dendritic cells in BrCa, except CHD3/5. The Kaplan–Meier Plotter analysis suggested that high expression levels of CHD1/2/3/4/6/8/9 were significantly related to shorter relapse-free survival (RFS), while higher mRNA expression of CHD1, CHD2, CHD8, and CHD9 was significantly associated with longer overall survival of BrCa patients. The miRNAs of hsa-miR-615-3p and hsa-let-7b-5p were identified as being more correlated with the CHD family. **Conclusion:** The altered expression of some CHD members was significantly related to clinical cancer outcomes, and CHD1/2/8/9 could serve as potential prognostic biomarkers to improve the survival of BrCa patients. However, to evaluate the studied CHD members in detail are needed further investigations including experimental validation.

Keywords: Chromodomain-helicase-DNA-binding proteins- CHD, breast tumor- breast cancer- prognostic biomarker

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Introduction

Cancer ranks as a leading cause of death and a major barrier to increasing life expectancy in countries [1]. Among cancer types, breast cancer (BrCa) is the most common type of malignant neoplasms [2, 3] and is the primary cause of mortality among women aged 45–55 years [4]. In addition, it is the second leading cause of cancer-induced death. Rahib et al. estimated that the most common cancer in 2040 will be breast (364,000 patients) [5]. Early diagnosis of breast cancer can lead to a better prognosis and increase the survival rate among patients. Several risk factors can enhance the possibility of breast cancer development [6]. Recent studies have found that epigenetic factors at the chromatin may regulate tumorigenesis, plasticity, and heterogeneity of tumor cells in breast cancer [7, 8]. Identifying these factors and related signaling pathways could be useful in discovering potential candidates for anticancer drugs [9, 6]. One of these epigenetic mechanisms is chromodomain-helicase-DNA-binding proteins. There are many families of ATP-dependent chromatin-remodeling enzymes [10]; among them is the family of chromodomain-helicase/ATPase-DNA-binding domain (CHD) proteins

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[11, 12]. CHDs are defined as a family of large proteins that exist as monomers or constituents of multimeric complexes, which have specific functions in various cell types and developmental stages [13]. The CHD family has nine members, which are divided into three subfamilies, which are subfamilies I (CHD1 and CHD2), subfamily II (CHD3/Mi2, CHD4, and CHD5), and subfamily III (CHD6, CHD7, CHD8, and CHD9) [12]. Abnormal histones or covalent modifications in DNA potentially disrupt gene expression, and chromatin remodeling can play a role in tumorigenesis [14]. The CHD proteins belong to the ATP-dependent chromatin remodeler complexes family, contributing to chromatin modification and gene regulation [15]. Emerging data have shown that subfamily I and II from CHD proteins act as potential tumor suppressors, and their inactivation is involved in the development of several types of human cancers [14, 16, 17]. In addition, studies have revealed that CHD family proteins contribute to drug resistance to therapy [18]. Recent studies have indeed suggested a link between CHD4 [9] and CHD5 [19, 20] with cancers, indicating their potential as biomarkers. For example, the down-regulation of CHD5, possibly mediated by promoter methylation, has been implicated in the development and progression of human BrCa [19]. However, the roles of CHD modulators in BrCa are not fully understood. Bioinformatics analysis has become an increasingly popular method among researchers for identifying novel diagnostic, prognostic, and treatment biomarkers in various types of human cancers [21, 22, 8, 23]. Therefore, the lack/poor of information about a link between CHD family genes and BrCa prompted us to investigate the roles of CHD family genes to discover potential prognostic markers through analysis of CHD family expression in BrCa by mining UALCAN, TIMER and Kaplan-Meier plotter databases. However, we explored the relationship between CHD family members and immune infiltration abundance in BrCa.

Materials and Methods

Data collection

Clinical features of the breast cancer specimens were acquired from the Cancer Genome Atlas (TCGA_ data portal. Samples were excluded based on the presence of any of the subsequent attributes: male sex, previous history of breast cancer disease, receipt of neoadjuvant therapy, and absence of documented data.

Evaluation of Prognostic Values of CHD Family

The Kaplan-Meier plotter (http://kmplot.com/analysis) [24] was utilized for the assessment of the prognostic value of CHD family member expression, with a focus on overall survival (OS) and relapse-free survival (RFS) in the BrCa patients. The log-rank p-value < 0.05 was a common cutoff for statistical significance.

Relationship of CHD Expression with Clinicopathological Characteristics of BrCa

The association of CHD expression with clinicopathological characteristics of BrCa such as human epidermal growth factor 2 (HER2), progesterone receptor (PR), tissue age nature, nodal status, estrogen receptor (ER) using bc-GenExMiner v4.8 (http://bcgenex.ico.unicancer.fr/BC-GEM) provided valuable insights into the potential role of CHD proteins in disease progression and patient outcomes. [25].

The CHD mRNA expression difference in BrCa patients was assessed by Dunnett-Tukey-Kramer's and Welch's tests, and $p < 0.05$ was considered remarkably significant.

mRNA Expression Analysis of CHD Family

GEPIA (http://gepia.cancer-pku.cn/) developed at Peking University, is a valuable tool for analyzing RNA sequence expression statistics of normal and tumor tissue samples [26]. It's particularly useful for differential mRNA expression analysis of CHD family members in gene expression between normal and tumor tissues. The p-value cutoff was considered significantly less than 0.05.

Transcriptional Expression of the CHD Family in BrCa

We have used UALCAN (http://ualcan.path.uab.edu/analysis.html) to study the relative transcriptional expression of the CHD family in various stages of BrCa [27]. This database provides studies according to The Cancer Genome Atlas (TCGA). Again, a $P < 0.05$ was known as statistically significant.

Relationship of CHD Expression with Immune Infiltrating Cells

We used the TIMER (https://cistrome.shinyapps.io/timer/) [28] database to confirm the relationship of CHD expression with immune infiltrating cells (neutrophils, macrophages, B cells, CD4+ T cells, CD8+ T cells, and Dendritic Cell).

Gene Enrichment Analysis

Enrichr (http://amp.pharm.mssm.edu/Enrichr) is a comprehensive resource for curated gene sets and a search engine that accumulates biological knowledge for further biological discoveries [29], which can help identify the enrichment of CHD families and related neighbor genes. The "Functional enrichment analysis," can provide insights into the molecular functions (MF) and biological processes (BP) associated with CHD members (P-value < 0.05 was significant).

Protein expression patterns of CHDs in BrCa

The Human Protein Atlas (https://www.proteinatlas.org/about/licence) is a website that includes immunohistochemistry-based expression information by integration of numerous omics technologies, counting mass spectrometry-based proteomics, system biology, antibody-based imaging, and transcriptomics [30]. This is being used to compare the protein expression of CHD family members between normal and tumor BrCa tissues by immunohistochemistry image. This could potentially highlight any significant differences or patterns that might be relevant to the progression or treatment of BrCa.

Identification of key miRNAs related to CHD member's family

MiRTarBase (https://miRTarBase.cuhk.edu.cn/) acts as a potent tool that will help seek miRNA targets of high confidence for miRNA–target interactions (MTIs). This tool is used to identify microRNAs related to the CHD family.

Results

Expression patterns of nine CHD family members in patients with BrCa

We used the UALCAN resource to investigate the expression difference of nine CHD genes between normal and tumor tissue in patients with BrCa at transcriptional levels. Our results showed that the mRNA expression of CHD3/4/7 have increased in tumor tissue compared to normal tissue (CHD3, p=<0.0001; CHD4, p=<0.0001; CHD7, P=<0.0001), whereas CHD2/5/9 mRNA levels were lower (CHD2, p=<0.0001; CHD5, p=0.003; and CHD9, p=<0.0001). The fact that CHD1/6/8 was not significant differences in their transcriptional levels between BrCa and normal tissues. The results are shown in Figure 1.

Correlation of mRNA levels of CHDs with clinicopathological characteristics of BrCa patients

The association of the mRNA expression of CHDs with clinicopathological characteristics of BrCa using bc-GenExMiner indicated that the higher SBR (Scarff-Bloom-Richardson) grade correlated with lower mRNA levels of CHD1/2/3/6/8/9 and a higher CHD7 (p< 0.0005). As indicated in Table 1, we identified a downregulation of CHD1 (p= 0.0430), CHD3 (p= 0.0052), and CHD6 (p= 0.0020) expression in the younger age group (<51 years old group) compared to the older age group (>51 years old), and also the lower mRNA level of CHD7 in the older age group (p= 0.0139), might indicate age-specific roles of these CHDs in BrCa. According to the results in Table 1, the mRNA level of CHD5 (p=0.0003) and CHD7 (p< 0.0001) was lower in ER & PR-positive BrCa compared to ER & PR-negative BrCa (p<0.0001), contrariwise, the mRNA levels of CHD1/2/3/6/8/9 were lower in ER & PR-negative group compared to ER & PR-positive. We also observed the downregulation of CHD1 (P=0.0313) and CHD6 (p=0.0081) in the negative nodal status group compared to the positive nodal status.

GO (Gene Ontology) enrichment analysis

GO Enrichment analysis of CHDs was obtained using the "Enrichr" package, and it analyzed the 100 correlated genes of CHDs for molecular function and biological process enrichment. These genes were found to have methylated histone binding (GO: 0035064; P= 1.70E-06), methylation-dependent protein binding (GO: 0140034; P=3.57E-05), and guanyl-nucleotide exchange factor activity (GO: 0005085; P= 9.80E-04) in terms of molecular function, significantly. In the biological process, the significant enrichment in the regulation of transcription, DNA template (GO: 0006357; p= 3.23E-08) for all CHD family members except CHD6/9 identified. Moreover, CHD1/2/3/4/5/8 was closely related to regulating transcription by RNA polymerase II (GO: 0006357; p=1.07E-05). The top 10 GO terms are shown in Figure 2 A, 2B.

Correlation of CHD expression in BrCa with infiltration of immune cells

In this study, the "Survival module" was used to evaluate the correlation between the abundance of immune infiltration and CHD gene expression using the TIMER database (Figure 3). There was a positive correlation between CHD1/2/4/6/7/8/9 and the infiltration of CD8+ T cells (CHD1: Cor = 0.271, p = 6.50e-18; CHD2: Cor = 0.321, p = 8.96e-25; CHD4: Cor = 0.217, p = 7.45e-12; CHD6: Cor = 0.249, p = 2.88e-15; CHD7: Cor = 0.2, p = 2.64e-10; CHD8: Cor = 0.296, p = 3.41e-21; and CHD9: Cor = 0.387, p = 2.91e-36), while CHD3/5 expression showed a negative correlation (CHD3: Cor = -0.016, p = 6.17e-01; and CHD5: Cor= -0.029, p = 3.69e-01. Figure 3). All nine CHDs were positively associated with the infiltration of CD4+ T cells (Figure 3). CHD1/3/6 expression negatively correlated with the infiltration of B cells (CHD1: Cor = -0.021, p = 5.18e-01; CHD3: Cor = -0.065, p = 2.26e-02; and CHD6: Cor = 0.017, p = 5.96e-01). Our analysis showed a positive correlation between CHD gene expression with macrophage, neutrophil, and dendritic cell infiltration in BrCa, except for CHD3/5. In the meantime, CHD2/8/9 strongly correlated with high infiltration of macrophage and neutrophil cells. CHD3 and CHD5 expression was negatively associated with the infiltration of neutrophil (Cor = -0.009, p = 7.76e-01) and macrophage (Cor = -0.03, p = 3.53e-01) cells, respectively (Figure 3).

Prognostic analysis of CHDs in patients with BrCa

To assess the prognostic value of CHD members in the BrCa, we evaluated the correlation between these genes' expression and clinical outcomes using the Kaplan–Meier database. It appears high expression level of CHD1/2/3/4/6/8/9 (CHD1: HR = 0.62, 95% CI: 0.53-0.72, P = 4.8e-10; CHD2: HR = 0.61, 95% CI:0.53-0.71, p = 1.8e-10; CHD3: HR = 0.85, 95% CI: 0.76–0.94, P = 0.0011; CHD4: HR = 0.8, 95% CI:0.72-0.89, p = 1.6e-05; CHD6: HR = 0.64, 95% CI:0.55-0.74, p = 4.4e-09; CHD8: HR = 0.81, 95% CI:0.73-0.9, p = 5.8e-05; and CHD9: HR = 0.78, 95% CI: 0.71–0.87, P=2.4e-06) were significantly related to shorter relapse-free survival (RFS) of BrCa patients (Figure 4).

On the other hand, higher mRNA expression of CHD1 (HR = 0.76, 95% CI: 0.63–0.92, P = 0.0041), CHD2 (HR = 0.68, 95% CI: 0.52–0.9, P = 0.0058), CHD8 (HR = 0.76, 95% CI: 0.63–0.92, P = 0.0045) and CHD9 (HR = 1.35, 95% CI: 1.03–1.77, P =0.029) was significantly associated with longer overall survival (OS) of BrCa patients (Figure 5). This indicates that these genes could potentially serve as prognostic biomarkers for BrCa patients' survival.

Protein expression patterns of CHDs in breast cancer

The protein expression patterns of CHDs using the Human Protein Atlas (HPA) database indicated that CHD2

Table 1. The Correlation between mRNA Level CHDs and Clinicopathological Features of BrCa Patients (bc-GenExMiner v4.2).

Criteria		Age		Nodal Status		ER(IHC)		PR(IHC)		HER2(IHC)		TNBrCa		BL-BrCa	
		≤51	>51	(-)	(+)	(-)	(+)	(-)	(+)	(-)	(+)	Not	TNBrCa	Not	BL
CHD1	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA		-		-		-		-	-	-	-		-	
	P-Value	0.043		0.0313		<0.0001		<0.0001		0.1219		<0.0001		<0.0001	
CHD2	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA	-	-	-	-		-		-	-		-		-	
	P-Value	0.1614		0.5957		<0.0001		<0.0001		<0.0001		0.0057		0.0005	
CHD3	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA		-	-	-		-		-	-		-		-	
	P-Value	0.0052		0.3858		<0.0001		<0.0001		0.008		<0.0001		<0.0001	
CHD4	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	P-Value	0.2042		0.5304		0.1058		0.1367		0.1637		0.2966		0.6783	
CHD5	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA	-	-	-	-	-		-		-	-		-	-	-
	P-Value	0.6325		0.3973		0.0003		0.0003		0.2865		0.0124		0.0611	
CHD6	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA		-		-		-		-	-	-	-		-	
	P-Value	0.002		0.0081		<0.0001		<0.0001		0.7148		<0.0001		<0.0001	
CHD7	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA	-		-	-	-		-		-	-		-		-
	P-Value	0.0139		0.3523		<0.0001		<0.0001		0.1527		<0.0001		<0.0001	
CHD8	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA	-	-	-	-		-		-	-	-	-		-	
	P-Value	0.2433		0.0503		<0.0001		<0.0001		0.3583		<0.0001		<0.0001	
CHD9	No.	267	476	332	358	187	530	243	470	396	109	578	87	605	136
	mRNA	-	-	-	-		-		-	-		-		-	
	P-Value	0.7522		0.3762		<0.0001		<0.0001		0.0415		0.0015		<0.0001	

was not expressed in BrCa tissue, while medium protein expressions of CHD3/4/9 were observed in tumor tissues. Low protein expression of CHD1 was observed in tumor tissues (Figure 6). These findings suggest that some CHDs are over-expressed in patients with BrCa, which could potentially have implications for understanding the disease progression and developing targeted therapies.

Identification of key miRNAs related to CHD member's family

In the present study, we identified miRNAs (microRNAs) related to the CHD family using the mirTarBase online database. Following database searching, ten miRNAs were identified as crucial miRNAs of the CHD family. As shown in Table 2, the detection of hsa-miR-615-3p and hsa-let-7b-5p as miRNAs with more correlation with the CHD family could open up new avenues for exploring the regulatory mechanisms in BrCa.

Table 2. Key miRNA of CHD Family

No.	Term	P-value	Genes
1	mmu-let-7b-5p	0.0003	*CHD9, CHD7, CHD3*
2	hsa-miR-615-3p	0.0004	*CHD8, CHD7, CHD5, CHD4*
3	hsa-miR-5189-3p	0.0007	*CHD7, CHD4*
4	hsa-miR-1193	0.0008	*CHD9, CHD4*
5	hsa-miR-1296-5p	0.0012	*CHD8, CHD3*
6	hsa-let-7b-5p	0.0013	*CHD7, CHD4, CHD3, CHD1*
7	hsa-miR-98-5p	0.0042	*CHD7, CHD4, CHD1*
8	hsa-miR-155-5p	0.0062	*CHD9, CHD8, CHD7*
9	mmu-miR-219a-5p	0.0067	*CHD6*
10	hsa-miR-450a-5p	0.0076	*CHD2*

Discussion

Recent research highlights the pivotal role of epigenetic modifications, specifically those involving histone and chromatin remodeling, in regulating gene expression and influencing cancer development. Among the key players in this context is the chromodomain helicase DNA-binding (CHD) family of chromatin remodelers [31], which can lead to abnormal gene expression and contribute to cancer progression. Some investigations have also suggested a correlation between the CHD family and the tumor microenvironment, which plays a crucial role in tumor progression and response to treatment. For instance, CHD7 mutations have been observed in small-cell lung cancers [32]. Additionally, CHD5 acts as a tumor suppressor, controlling cell proliferation and apoptosis pathways [31]. Evidence suggests that the CHD family is

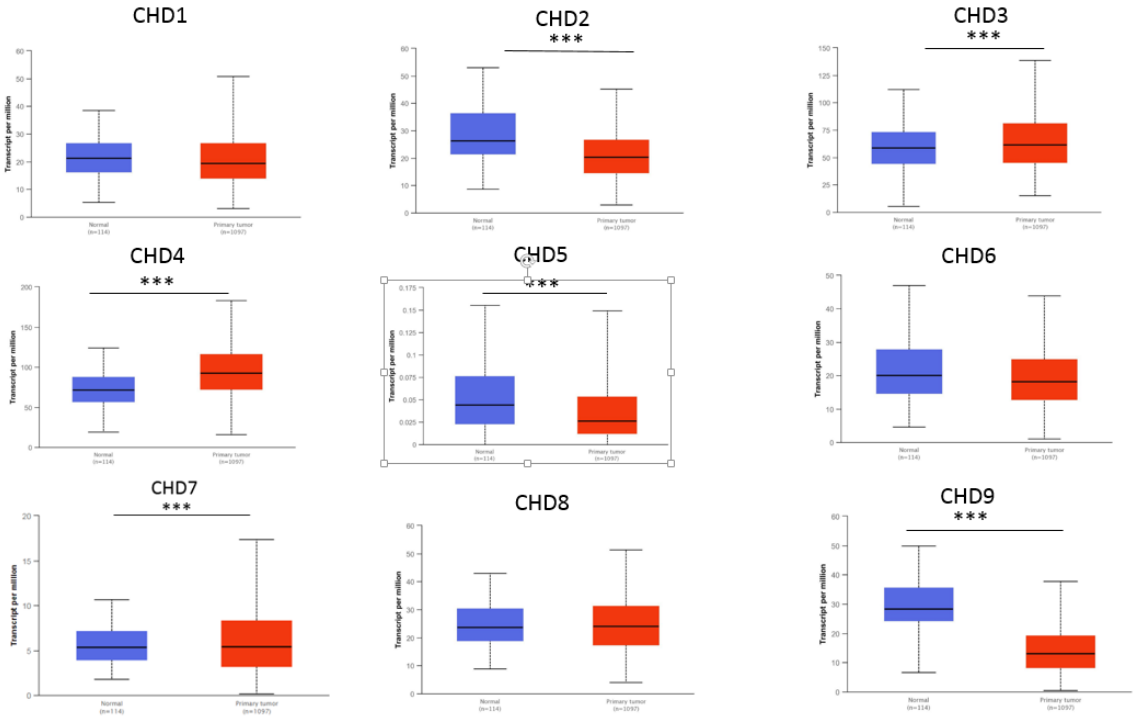

Figure 1. The Transcription of CHD Genes in BrCa (UALCAN). The transcriptional levels of CHD 3/4/7 in BrCa tissues were significantly elevated, while the transcriptional levels of CHD 2/5/9 were significantly reduced.

involved in various biological processes associated with cancer development, and also changes in CHD genes may potentially contribute to the initiation and progression of human cancer [33]. Despite the significance of the CHD family, its prognostic value in BrCa remains insufficiently described. So, we explored the prognostic implications and immune function of the CHD family members in BrCa patients.

From among the CHD family, the CHD1 protein plays a crucial role in directing lineage-specific transcription

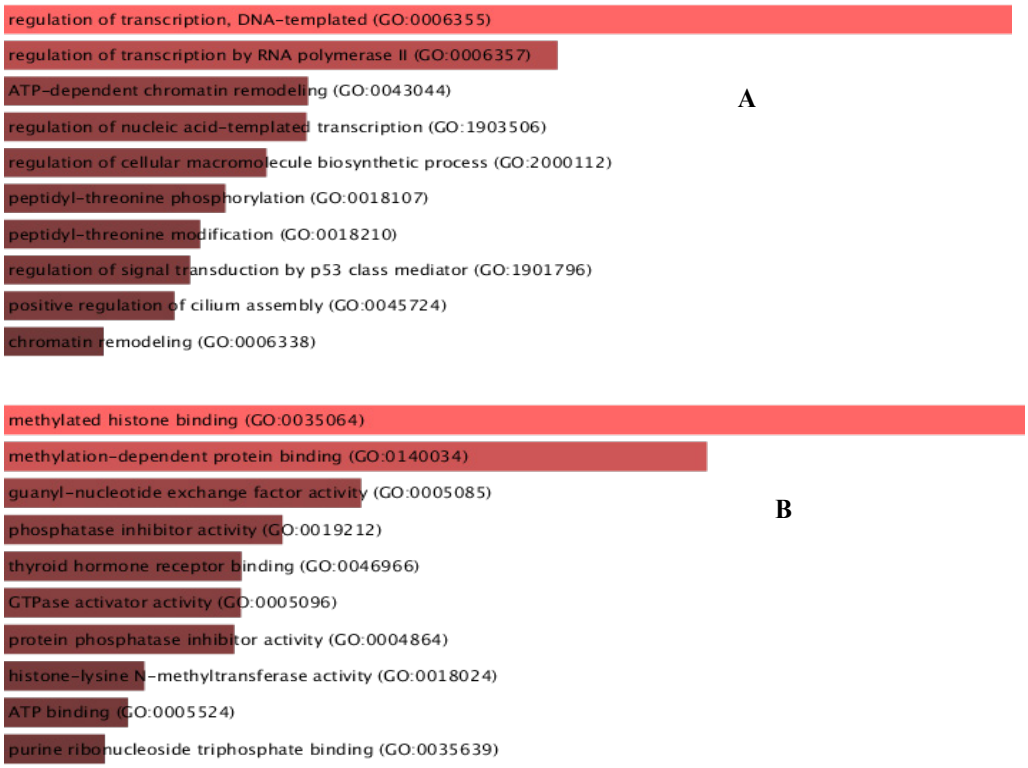

Figure 2. The Enrichment Analysis of the CHDs Family in BrCa. The GO Enrichment in Biological Process (A) and Molecular Function (B) Terms.

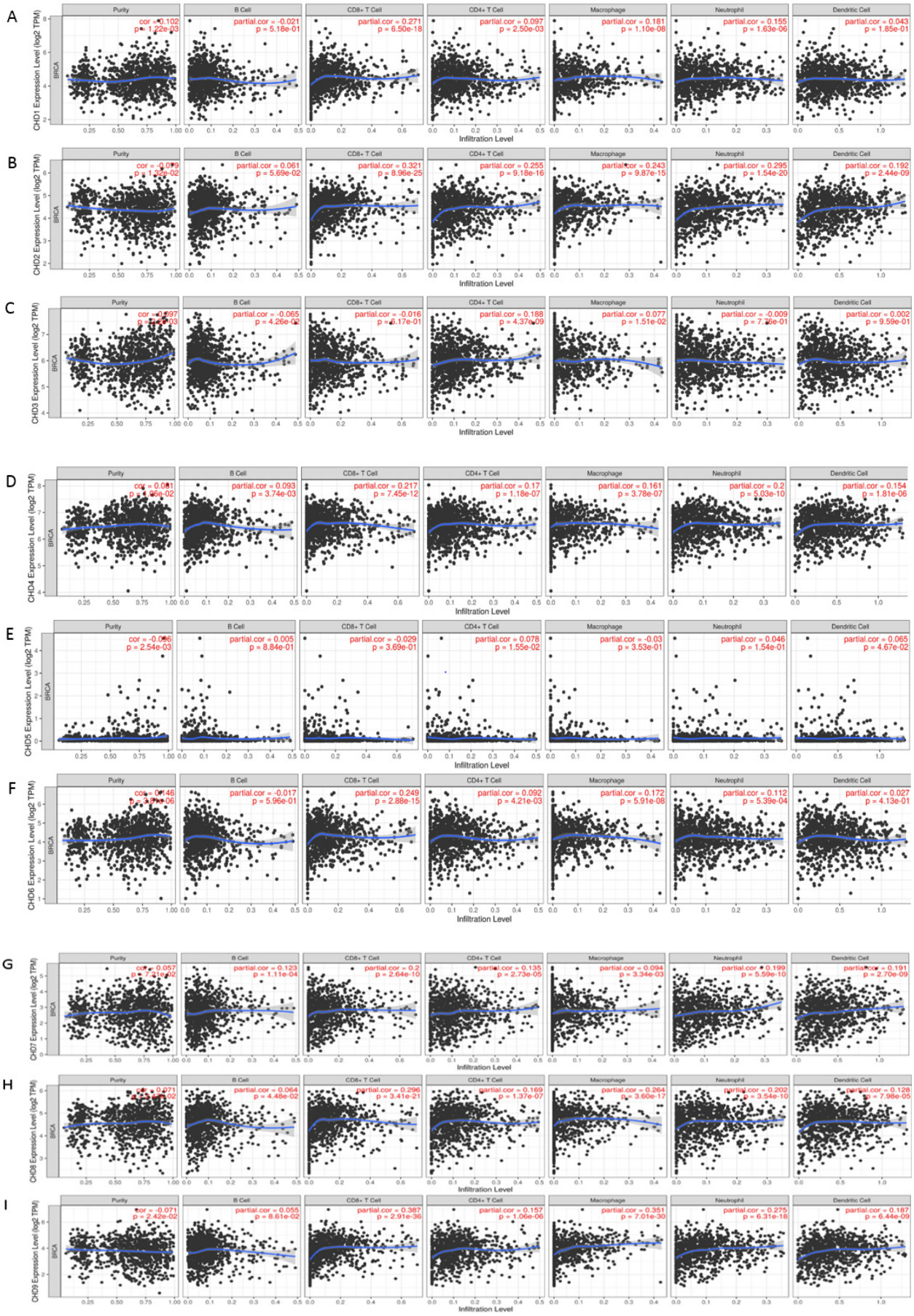

Figure 3. The Correlation between CHD Family and Immune Cell Infiltration by TIMER. The correlation between the abundance of immune cells and the expression of CHD1 (A), CHD2 (B), CHD3 (C), CHD4 (D), CHD5 (E), CHD6 (F), CHD7 (G), CHD8 (H), CHD9 (I) in BrCa.

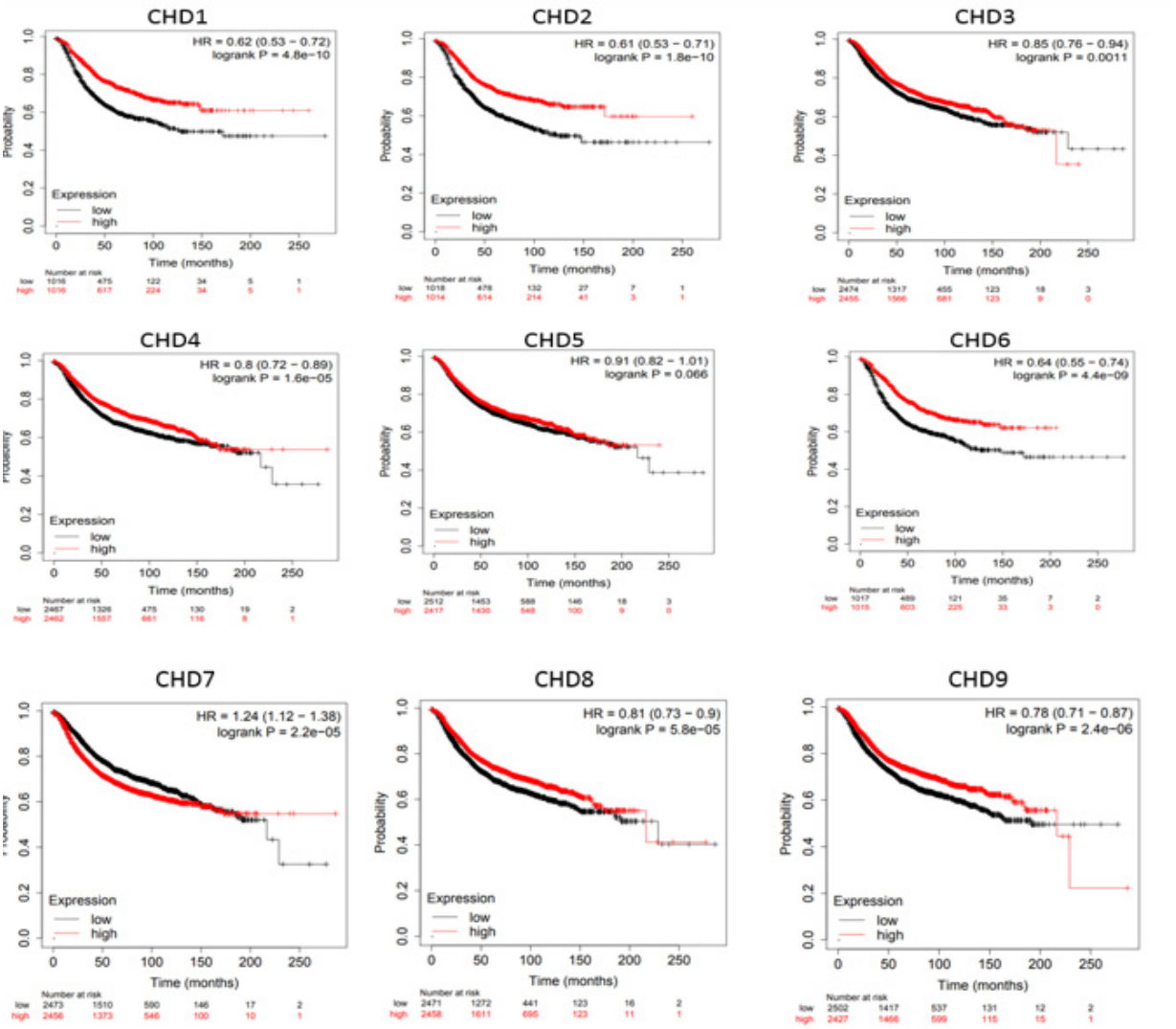

Figure 4. The Association of mRNA Expression of CHD Family with Relapse-Free Survival (RFS) of BrCa Patients (Kaplan-Meier Plotter).

and maintaining DNA regulatory regions in an actively transcribed state [34]. Specifically, CHD1 is essential for double-strand break (DSB) repair through homologous recombination. However, reduced CHD1 expression can lead to genomic instability and subsequent tumor development [35]. Numerous studies have explored CHD1's relevance in cancer patients, particularly prostate cancer. For instance, the deletion of CHD1 has been associated with cancer and postoperative biochemical relapse (BCR) in a cohort of prostate cancer (PCa) patients, suggesting it is a poor prognosis marker [36]. Recent research by Oh-Hohenhorst et al. revealed that CHD1 loss increases the risk of postoperative metastasis in R0-resected PCa patients and promotes spontaneous metastasis formation in vivo [37]. In a study investigating CHD1's potential roles in breast cancer, researchers introduced shRNA-mediated depletion of CHD1 into both PTEN-deficient and PTEN-intact breast cancer cell lines. The results indicated that CHD1 suppression inhibited the proliferation and tumor growth of PTEN-deficient breast cancer cells [38]. Despite this, accumulating evidence suggests that CHD1 acts as a tumor suppressor across a wide range of human cancers [39]. In our study, CHD1 expression did not significantly differ between BrCa tissues and normal tissues. However, low CHD1 expression correlated significantly with higher SBR grades. Surprisingly, higher mRNA expression of CHD1 was associated with longer OS in BrCa patients, which appears contradictory to the role of CHD1 as a tumor suppressor in other human cancer types.

Genomic evidence supporting the occurrence of CHD2 defects across various cancer types remains limited. However, a study aimed at understanding the functional role of CHD2 in mammals sheds light on its significance. Nagarajan et al. created a CHD2 mutant mouse model [40], and the authors propose that CHD2 plays a critical role in development, hematopoiesis, and tumor suppression. Interestingly, CHD2 heterozygous mutant mice exhibit elevated extramedullary hematopoiesis and are susceptible to lymphomas. At the cellular level, CHD2 mutants display defects in hematopoietic stem cell differentiation, accumulate higher levels of the chromatin-associated DNA damage response mediator, and exhibit an aberrant DNA damage response following X-ray irradiation [40].

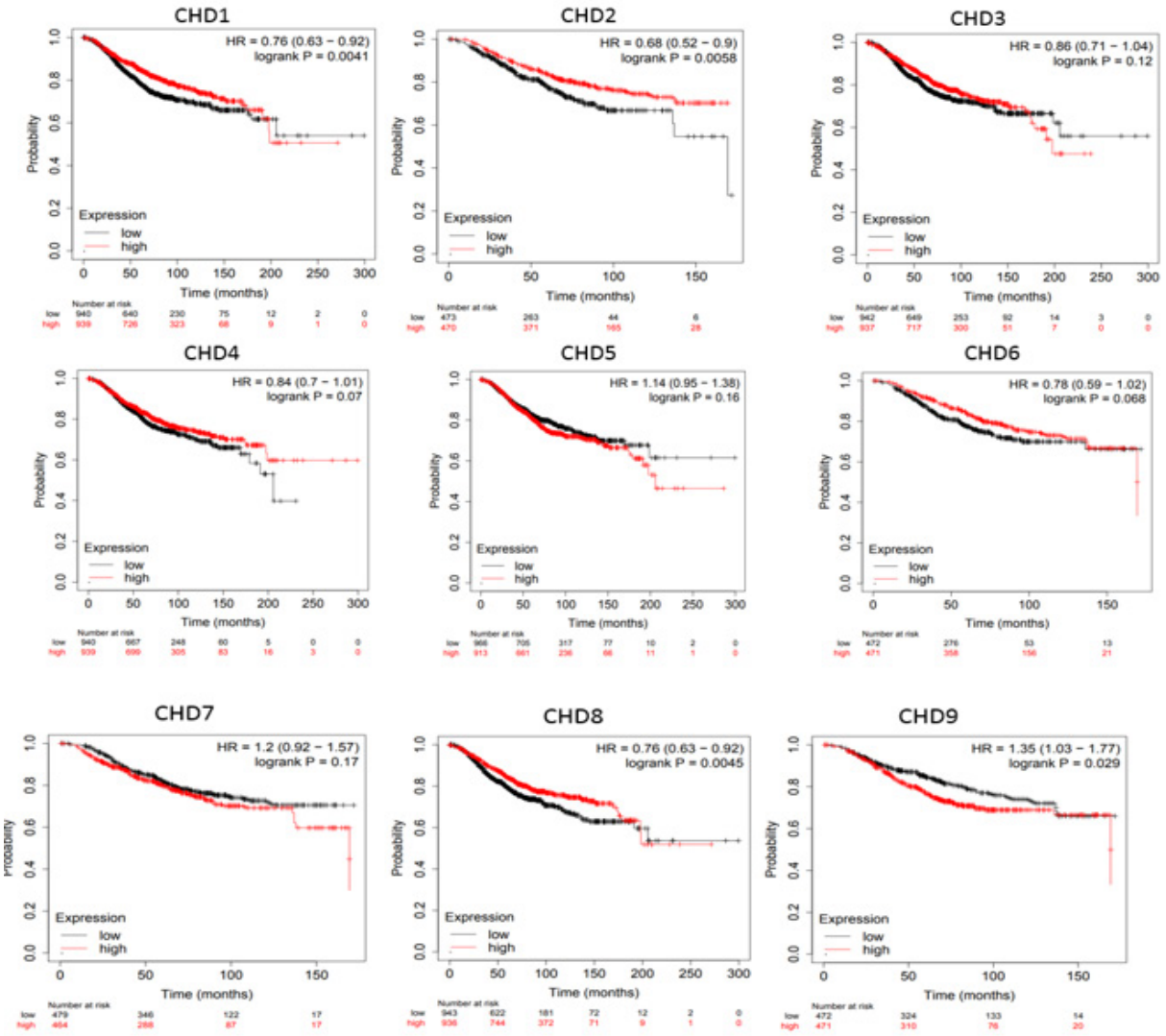

Figure 5. The Association of mRNA Expression of CHD Family with Overall Survival (OS) of BrCa Patients (Kaplan-Meier Plotter).

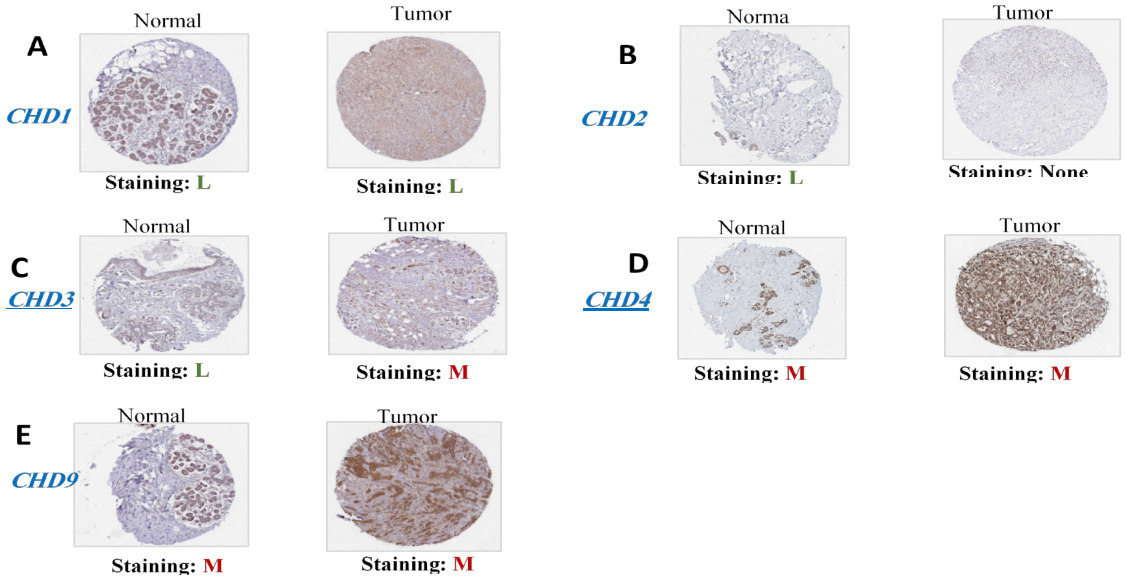

Figure 6. Representative Immunohistochemistry Images of CHD Family Members in BrCa and Normal Tissues (Human Protein Atlas Database). L: Low; M: Medium; None: Not detected

Additionally, female mice with heterozygous CHD2 mutant allele develop cystic endometrial hyperplasia [41]. In a separate study, Koch et al. identified CHD2 as a potential genetic modifier for mammary tumors in BALB/CJ mice heterozygous for p53 [42]. Our analysis reveals significantly reduced transcriptional levels of CHD2 in BrCa tissues. Notably, there is a positive correlation between CHD2 expression and the infiltration of CD8+ T cells, as well as an association with higher overall survival (OS) in BrCa patients. Based on these findings, CHD2 emerges as a promising biomarker for predicting the prognosis of breast cancer patients.

CDH3 is recognized as an oncogene in various malignancies [43]. According to a recent study, CHD3 expression exhibits alterations between gastric cancer (GC) samples and normal controls. Notably, it is remarkably associated with advanced-stage cancer and elevated expression of CHD3 along with CHD4/6/8 was found to be significantly correlated with poor OS and progression-free survival (PFS) [44]. In another study, CDH3 is up-regulated in oral squamous cell carcinoma (OSCC) samples and is closely linked to poor prognosis. Also, the knockdown of CDH3 reduced cell viability, impaired colony formation, compromised migration, increased invasion, and heightened chemo-resistance in OSCC cells. As a result, the authors suggest that CDH3 contributes to malignancy and chemo-resistance in OSCC [43]. In the present study, we observed downregulation of CHD3 expression in the <51 years old group compared to the >51 years old. Also, we found a negative correlation between CHD3 expression and the infiltration of CD8+ T and B cells. Furthermore, the high CHD3 expression is associated with shorter relapse-free survival (RFS) of BrCa patients. Based on these obtained results, further investigations are needed to assess the clinical utility of CHD3 members in BrCa patients. These findings warrant further investigations to assess the clinical utility of CHD3 as a potential biomarker in BrCa patients.

CHD4 is an epigenetic regulator and an oncogenic element with potential implications for novel therapeutic approaches in treating breast cancer. CHD4 and the NuRD complex are involved in gene regulation/or expression in normal and cancer cells [45]. Recent studies have focused on CHD4's role in gene activation, and it acts as a coactivator of hypoxia-inducible factors [46], thereby promoting breast cancer progression. Breast tumor initiation and progression predominantly result from acquired genetic alterations [47]. Novillo et al. explored the impact of CHD4 mutations in various types of BrCa, often occurring alongside mutations in tumor suppressor genes or oncogenes [9]. CHD4 also plays a role in regulating the Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2) signaling pathway and autophagy specifically in ERBB2-positive breast cancer cells [48]. In our study, we observed higher CHD4 expression in breast cancer tissues than in normal tissues. Surprisingly, low CHD4 expression was significantly correlated with poor overall survival (OS) in BrCa patients. Further functional analyses are required to fully elucidate the roles of CHD4 as a prognostic marker in BrCa patients.

CHD5 is thought to play a role in nucleosome remodeling and deacetylation through the formation of the NuRD complex, and it exerts control over specific gene transcription [20]. Notably, CHD5 functions as a tumor suppressor gene in various cancer types, including gliomas, breast, lung, colon, prostate, and ovarian cancers [49]. Research by Kolla and colleagues demonstrated that low CHD5 expression correlates strongly with negative biological and clinical characteristics in neuroblastomas and other tumor types [50]. Furthermore, Wu et al. indicated that reduced CHD5 expression, partly mediated by promoter methylation, contributes to the progression and development of human breast cancer [19]. Interestingly, our findings indicate a high expression level in CHD6/8/9 in terms of overall survival (OS) and recurrence-free survival (RFS).

CHD7, which exhibits significant amplification in over 5% of samples across eleven tumor kinds, including breast, ovarian, lung, and colorectal cancers [32], plays a pivotal role in stem cell differentiation and cell fate determination. In addition, novel mutations in CHD genes could lead to severe developmental conditions [51]. An intriguing finding suggests that SOX2 and CHD7 collaborate to regulate a select group of genes. These genes include Sonic Hedgehog and NOTCH pathway genes and classical oncogenes such as SRC and NRAS, their collective function is crucial in stem cell tumorigenesis and development [52]. Research by Colbert et al. indicated that CHD7 expression serves as a predictor for survival outcomes in patients with resected pancreatic cancer. Interestingly, low CHD7 expression has been associated with improved recurrence-free survival (RFS) and overall survival (OS) in patients receiving adjuvant gemcitabine treatment [53]. CHD9 is the lesser-explored considered subfamily member and acts as a transcriptional regulator in mesenchymal stem cells [54] and rat liver [55]. Its interactions with nuclear receptors contribute to glucocorticoid receptor-mediated gene expression control and also can contribute to loosening the chromatin structure in animal models [56].

In addition to analyzing the mRNA expression levels of the CHD family, we also identified key miRNAs associated with these genes. In this regard, miR-615-3p and has-let-7b-5p emerged as the most relevant miRNAs concerning the CHD family. Growing evidence suggests that miRNAs play a crucial role in regulating epithelial-mesenchymal transition (EMT), a process implicated in cancer development. Notably, miR-615-3p has been found to be significantly upregulated in breast cancer (BrCa) cells and tissues, particularly in the metastatic form [57]. This heightened expression of miR-615-3p appears to promote EMT and metastasis in BrCa, underscoring its pivotal role in the pathophysiology of the disease [57]. Recently, another miRNA, let-7b-5p has garnered attention for its multifaceted role in regulating tumorigenesis and cancer progression. Notably, Let-7b-5p exerts inhibitory effects on BrCa cell growth and metastasis by repressing hexokinase 2-mediated aerobic glycolysis [58]. These findings collectively highlight the potential therapeutic application of miRNAs in the context of breast cancer therapy.

In conclusion, we aimed to evaluate the expression

and prognostic value of CHDs in breast cancer. Our analysis presented that the changed expression of certain CHD members significantly correlates with clinical cancer outcomes in BrCa patients. Specifically, we observed increased mRNA levels of CHD3/4/7 in breast cancer tissues compared to normal tissues. Conversely, CHD2/5/9 exhibited decreased expression in tumor tissues. Our analyses also highlighted a positive correlation between CHD gene expression and infiltration of macrophages, neutrophils, and dendritic cells in BrCa, except for CHD3/5. Furthermore, Kaplan–Meier Plotter analysis demonstrated that high expression levels of CHD1/2/3/4/6/8/9 were significantly associated with shorter relapse-free survival (RFS) in breast cancer patients. Conversely, elevated mRNA expression of CHD1, CHD2, CHD8, and CHD9 was significantly linked to longer overall survival (OS) in the same patient group. These findings suggest a prognostic value for CHD1/2/8/9 in breast cancer patients' survival. Additionally, we identified hsa-miR-615-3p and hsa-let-7b-5p as key miRNAs targeting the most CHDs within the family. These miRNAs hold promise for survival management in BrCa patients.

However, it's essential to acknowledge that our study had limitations, including the reliance on data extracted from online databases. Further investigations are warranted to evaluate the clinical data related to the studied CHD members in greater detail.

Author Contribution Statement

All authors contributed equally in this study.

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Ethics approval and consent to participate

Ethical approval and informed consent are not necessary for this study.

Conflict of interest

The authors declare that they have no conflict of interest.

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