European Journal of Biology
Eur J Biol 2024; 83 (1): 19–27
DOI: 10.26650/EurJBiol.2024.1362117

RESEARCH ARTICLE

Retrospective Analysis of Transcriptomic Differences between Triple-Negative Breast Cancer (TNBC) and non-TNBC

Caglar Berkel[1]

[1]Tokat Gaziosmanpasa University, Faculty of Science and Arts, Department of Molecular Biology and Genetics, Tokat, Türkiye

ABSTRACT

Objective: Triple-negative breast cancer (TNBC), which has no expression of estrogen receptor, progesterone receptor and HER2, is an aggressive subgroup. Molecular differences between TNBC and non-TNBC should be better understood to develop tailored treatment strategies.

Materials and Methods: The expression of the most frequently mutated genes, and of genes for which copy number variation events are observed in the highest percentage of breast cancer patients, was compared between TNBC and non-TNBC samples, in R programming environment, using TCGA-BRCA transcriptomics dataset.

Results: 70% of the most frequently mutated genes in breast cancer (*CDH1, GATA3, MLL3 (KMT2C), MAP3K1, PTEN, NCOR1, FAT3, MAP2K4, NF1, ARID1A, LRP1B, RUNX1, MLL2 (KMT2D)* and *TBX3*) was found to have decreased expression in TNBC compared to non-TNBC. The expression of 40% of the genes with the highest frequency of copy number gain events in breast cancer (*SLC45A3, PTPRC, ELF3, FCGR2B, AKT3, FH, TPM3* and *SETDB1*) was increased in TNBC compared with non-TNBC. The half of the genes with the highest percentage of copy number loss events in breast cancer (*CBFA2T3, CDH1, ZFHX3, CDH11, MAP2K4, GAS7, PER1, RABEP1, NCOR1* and *PCM1*) was observed to have decreased expression in TNBC compared to non-TNBC. Lastly, the expression of BRCA2, but not of BRCA1, was found to be higher in TNBC than in non-TNBC.

Conclusion: This study provides further evidence in support of previous research, which show the presence of a large number of molecular differences between TNBC and non-TNBC, pointing to the need for more tailored treatment strategies for patients with TNBC.

Keywords: Breast cancer; Estrogen receptor; Progesterone receptor; HER2; Copy number variation; Transcriptomics; Triple-negative breast cancer.

INTRODUCTION

Breast cancer is the most common cause of cancer-associated deaths in female patients, with an estimate of more than 2,000,000 new cases and approximately 700,000 deaths each year worldwide.[1,2] This malignancy has been classified into different subgroups, mainly based on the presence/absence of the expression of three receptors: estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor (HER2/ERBB2). Despite the presence of high levels of heterogeneity at the molecular and cellular levels in breast tumors, the majority of the patients with breast cancer are treated with untailored therapies with certain chemotherapeutics or hormone therapies, including tamoxifen, a selective ER modulator, neglecting the molecular diversity and heterogeneity between the subgroups of the disease. Therefore, there is an immediate need to develop novel targeted therapy modalities that are matched to the particular molecular and cellular changes in a breast tumor, with the ultimate purpose of achieving improved treatment benefits and avoiding excessive therapy.[3]

Triple-negative breast cancer (TNBC), which does not have hormone receptor (ER and PR) and HER2 expression, is an aggressive subtype of breast cancer for which novel therapy strategies need to be developed.[4,5] TNBC represents around 10–15% of all tumors of the breast, with an unfavorable prognosis at the clinic compared with non-TNBC.[6-9] Using current standard treatment options, the median overall survival for patients with TNBC is around 10.2 months. The 5-year survival rate is approximately 65% for patients whose tumors have spread to nearby lymph nodes, local tissues, or organs, and 11% for patients whose disease has metastasized from

Corresponding Author: Caglar Berkel **E-mail:** caglar.berkel@gop.edu.tr
Submitted: 18.09.2023 • **Revision Requested:** 25.10.2023 • **Last Revision Received:** 22.11.2023 • **Accepted:** 23.11.2023 • **Published Online:** 31.01.2024

breast tissue to distant organs in the body.[10,11] In addition to the aggressive characteristics of this subtype, the limited targeted therapy options and insensitivity to endocrine agents contribute significantly to poor disease-free and overall survival in this patient group.[6,9] Although therapeutic strategies such as immune checkpoint inhibitors and PARP (Poly (ADP-ribose) polymerase) inhibitors (also known as PARPi) including olaparib are changing the treatment landscape[12], TNBC currently has the worst prognosis among all breast cancer subtypes. This indicates an urgent need for a more complete understanding of TNBC which might help researchers develop therapeutic strategies with higher efficacy for patients with this subtype of breast cancer.

In the present study, molecular differences at the transcript level between TNBC and non-TNBC were studied. Between TNBC and non-TNBC samples, the expression of the most frequently mutated genes and of genes for which copy number variation (CNV) events (gain and loss) are observed in the highest percentage of breast cancer patients, were compared to identify essential genes that possibly contribute to clinical differences between TNBC and non-TNBC. Seventy percent of the most frequently mutated genes (*CDH1, GATA3, MLL3 (KMT2C), MAP3K1, PTEN, NCOR1, FAT3, MAP2K4, NF1, ARID1A, LRP1B, RUNX1, MLL2 (KMT2D)* and *TBX3*) in breast cancer was found to have decreased expression in TNBC compared to non-TNBC. Forty percent of the genes with the highest frequency of copy number gain events in breast cancer (*SLC45A3, PTPRC, ELF3, FCGR2B, AKT3, FH, TPM3* and *SETDB1*) was shown to have increased expression in TNBC compared with non-TNBC. The half of the genes with the highest frequency of copy number loss events in breast cancer (C*BFA2T3, CDH1, ZFHX3, CDH11, MAP2K4, GAS7, PER1, RABEP1, NCOR1* and *PCM1*) was observed to have decreased expression in TNBC compared to non-TNBC. Lastly, the expression of *BRCA2*, but not of *BRCA1*, was found to be higher in TNBC than in non-TNBC. This study points to the presence of many molecular differences between TNBC and non-TNBC at the expression level of genes of clinical importance, pointing to the need for more tailored treatment strategies for breast cancer patients with triple-negative status.

MATERIALS AND METHODS

Datasets

In the present study, mutation percentage and copy number variation (CNV) data were obtained from the Genomic Data Commons (GDC) Data Portal of The National Cancer Institute, which can be accessed at https://portal.gdc.cancer.gov/, which includes data from TCGA (The Cancer Genome Atlas)-BRCA project in addition to other projects.[13-17] The most frequently mutated genes were defined as genes with the highest percentage of cases affected by mutations in these genes (for instance, 442 out of 1387 patients with breast cancer have a mutation in the *TP53* gene, the most frequently mutated gene in breast cancer). Only the top 20 most commonly mutated genes were included in this analysis.

For transcriptomic analysis, processed and compiled RNA sequencing and clinical data for breast cancer patient samples from the TCGA project (GSE62944) were used.[18-20] In more detail, in the construction of datasets, authors aligned the fastq files.[18] First, they aligned the reads with the align function to the UCSC hg19 reference genome. Second, they used the featureCounts function to summarize the gene expression values as integers. Lastly, these summarized gene values were normalized to FPKM and TPM values.[18] The total sample size (n) for the number of patients after the filtering steps is 703. Sample sizes (i.e., number of patients) for subgroups are as follows: non-TNBC (non-triple negative breast cancer): 591; TNBC (triple-negative breast cancer):112. TNBC is defined as ER- (estrogen receptor-negative), PR- (progesterone receptor-negative), and HER2- (human epidermal growth factor receptor 2-negative). This dataset can also be accessed in SummarizedExperiment format through Bioconductor (in Experiment Packages » GSE62944).[21-23] This dataset also includes clinical variables for patients other than those used in the present study. Raw data for this dataset can be found at GEO using the given accession ID. In this dataset, ER, PR, and HER2 status from patient breast tumor samples were determined using immunohistochemistry.[19]

Data Analysis and Visualization

Analysis and visualization of the obtained data in the present study were conducted in R statistical programming environment (R version 4.2.1 (2022-06-23)) using R Studio IDE from posit.[24] These R/Bioconductor packages (https://bioconductor.org/) were used throughout the analysis[22,23]: tidyverse (a collection of R packages written for data science applications including ggplot2 and tidyr)[25-27], readxl[28], ExperimentHub[29], SummarizedExperiment[30], ggpubr[31] (for statistical tests), rmarkdown[32] and knitr.[33] After processing, TCGA gene expression data (GSE62944) was accessed using the ExperimentHub package for all cancer types with the query function from the AnnotationHub package; as the "CancerType" variable, "BRCA" was selected (CancerType== "BRCA"), which is short for breast cancer. R script used in the analysis is available as a supplementary document for reproducibility purposes.

The Wilcoxon test was performed when the expression data was not normally distributed.[31] Otherwise (when we can assume normality, i.e., p-value> 0.05 for Shapiro-Wilk test of normality), the *t-test* was used in the comparison of group means. Functions (ggqqplot for quantile-quantile plot and shapiro.test for Shapiro-Wilk normality test) from stats and ggpubr packages were used in the analysis of the distribution (normal dis-

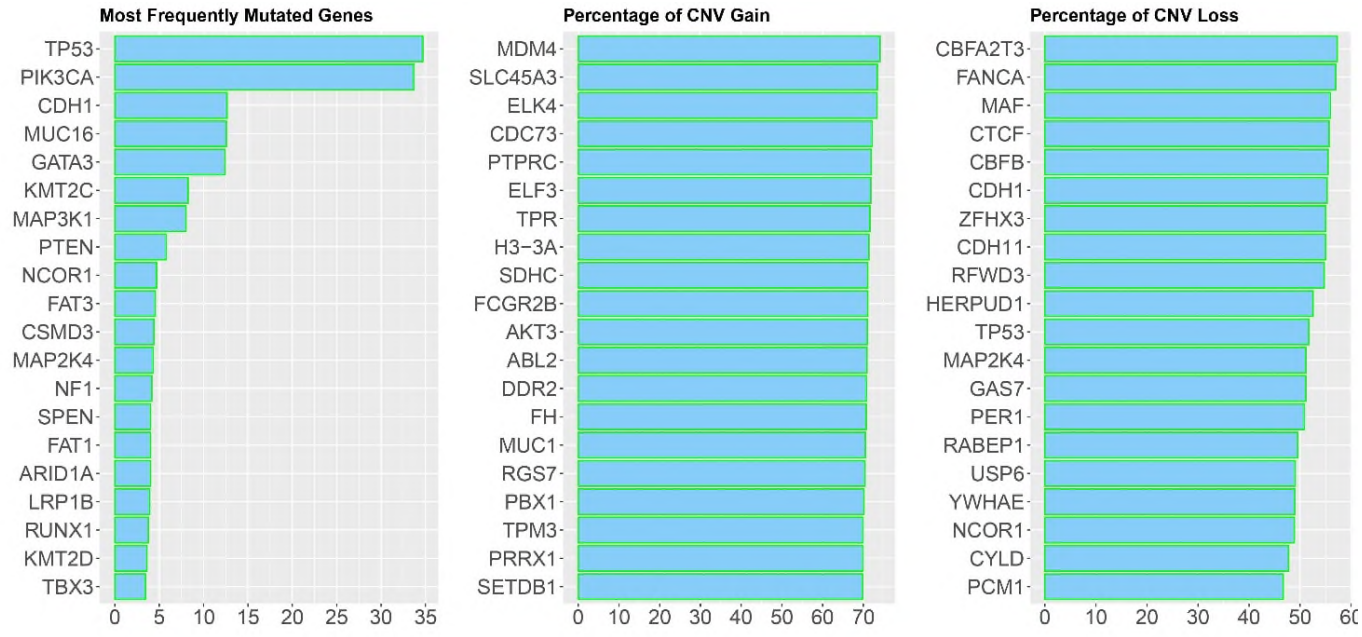

Figure 1. The list of the top 20 genes with the highest percentage of mutations (first panel), the highest percentage of copy number gain events (middle panel), or copy number loss events (last panel) in breast cancer. The x-axis in the first panel shows the number of cases in which a gene is mutated divided by the number of cases investigated for the presence of simple somatic mutations. CNV: copy number variation.

tribution or not).[24] Relative expression values shown in plots are log10 transformations of read counts from the dataset. Data analysis and visualization were performed as reported in our previous studies.[34,35]

RESULTS

Seventy Percent of the Most Frequently Mutated Genes in Patients with Breast Cancer Shows Decreased Expression in TNBC compared to Non-TNBC.

Firstly, the transcript levels of the top 20 most frequently mutated genes in breast cancer (namely, *TP53, PIK3CA, CDH1, MUC16, GATA3, MLL3, MAP3K1, PTEN, NCOR1, FAT3, CSMD3, MAP2K4, NF1, FAT1, SPEN, ARID1A, LRP1B, RUNX1, MLL2* and *TBX3*) (Figure 1) were compared between tumors from breast cancer patients with triple negative status (ER-, PR-, HER2-) and non-TNBC. Fourteen genes out of these 20 genes (70%) were found to have decreased expression in TNBC compared to non-TNBC (Figure 2). These 14 genes are: *CDH1, GATA3, MLL3 (KMT2C), MAP3K1, PTEN, NCOR1, FAT3, MAP2K4, NF1, ARID1A, LRP1B, RUNX1, MLL2 (KMT2D)* and *TBX3* (Figure 2). In contrast, PIK3CA, MUC16 and FAT1 showed increased expression in TNBC compared to non-TNBC (Figure 2). TP53, CSMD3, and SPEN expression did not change between patients with TNBC and non-TNBC at the transcript level (Figure 2).

Forty Percent of the Genes with the Highest Percentage of CNV Gain Events in Breast Cancer Shows Increased Expression in TNBC compared to Non-TNBC.

Next, mRNA levels of the top 20 genes for which the highest percentage of CNV gain events are observed in patients with breast cancer (namely, *MDM4, SLC45A3, ELK4, CDC73, PTPRC, ELF3, TPR, H3-3A (H3F3A), FCGR2B, SDHC, AKT3, ABL2, DDR2, FH, MUC1, RGS7, PBX1, PRRX1, TPM3* and *SETDB1*) (Figure 1), were compared between tumors from patients with TNBC and non-TNBC (Figure 3). The expression of 5 genes (*MDM4, MUC1, RGS7, PBX1*, and *PRRX1*) (25%) was found to be decreased in TNBC compared to non-TNBC (Figure 3). In contrast, the expression of *SLC45A3, PTPRC, ELF3, FCGR2B, AKT3, FH, TPM3*, and *SETDB1* (8 genes out of 20; 40%) was shown to be higher in breast tumors with triple-negative status than in those with non-triple negative status (Figure 3). The other seven genes did not show significantly different expression between patients with TNBC and non-TNBC (Figure 3).

Half of the Genes with the Highest Percentage of CNV Loss Events in Breast Cancer Shows Lower Expression in TNBC than in Non-TNBC.

Then, the expression levels of the top 20 genes for which the highest percentage of CNV loss events are observed in breast cancer patients (that are *CBFA2T3, FANCA, MAF, CTCF, CBFB, CDH1, ZFHX3, CDH11, RFWD3, HERPUD1, TP53, MAP2K4, GAS7, PER1, RABEP1, USP6, YWHAE, NCOR1,*

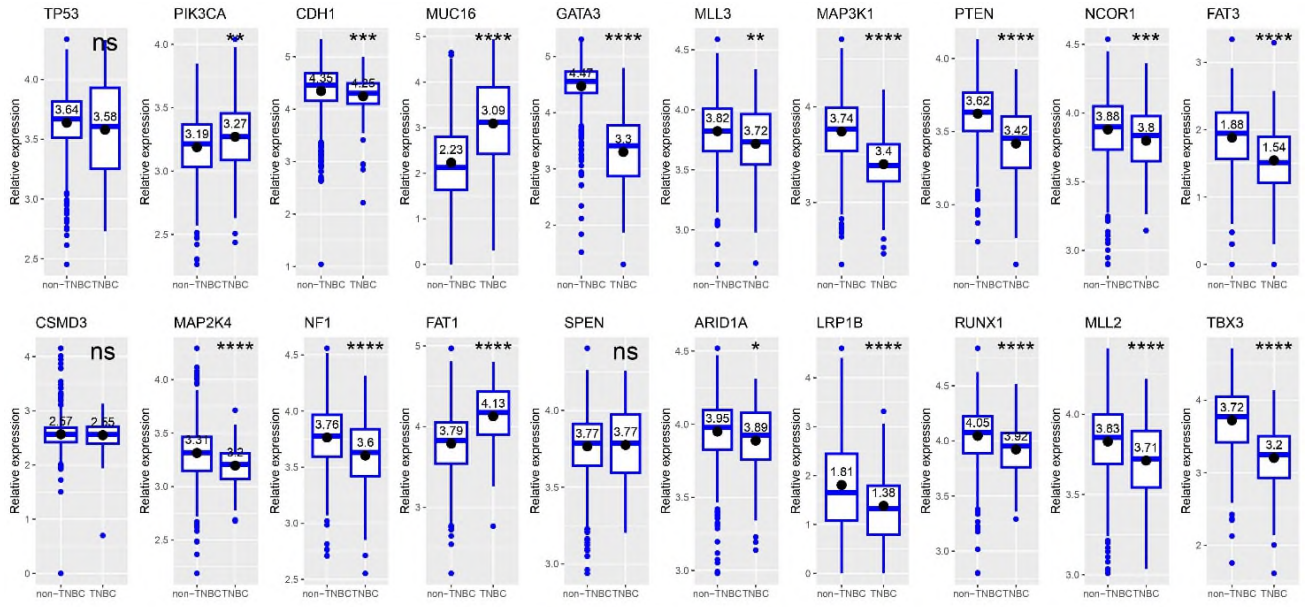

Figure 2. Comparative expression of the most frequently mutated genes in breast cancer between triple-negative breast cancer (TNBC) and with non-TNBC. The following convention of star symbols for statistical significance was used in the comparison of group means: ns (non-significant): $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

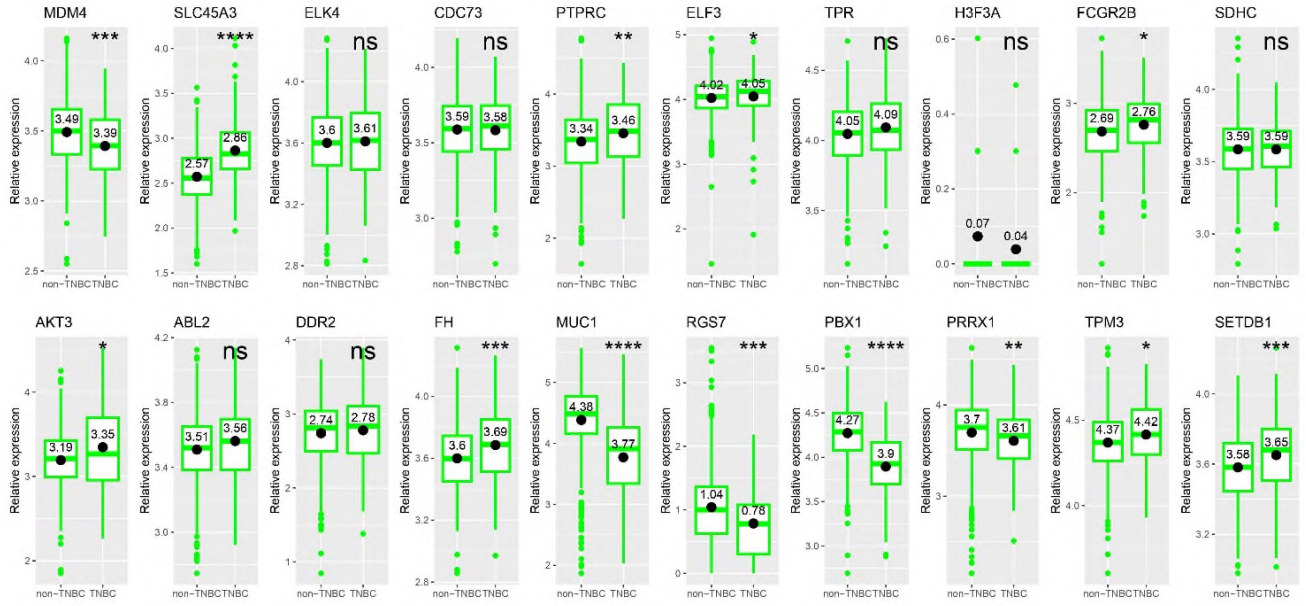

Figure 3. Comparative expression of the genes with the highest percentage of copy number gain events in breast cancer between triple-negative breast cancer (TNBC) and non-TNBC. The following convention of star symbols for statistical significance was used in the comparison of group means: ns (non-significant): $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

CYLD and *PCM1*) (Figure 1), were comparatively analyzed between tumors from patients with TNBC and non-TNBC (Figure 4). The half of these genes (*CBFA2T3, CDH1, ZFHX3, CDH11, MAP2K4, GAS7, PER1, RABEP1, NCOR1* and *PCM1*) was observed to have decreased expression in TNBC compared to non-TNBC (Figure 4). *FANCA, CBFB, RFWD3*, and *YWHAE* expression were higher in breast tumors with triple-negative status than in those with non-TNBC (Figure 4). The other six genes (30%) (*MAF, CTCF, HERPUD1, TP53, USP6*, and *CYLD*) did not exhibit differential expression in breast cancer based on triple negativity status (TNBC vs non-TNBC) (Figure 4).

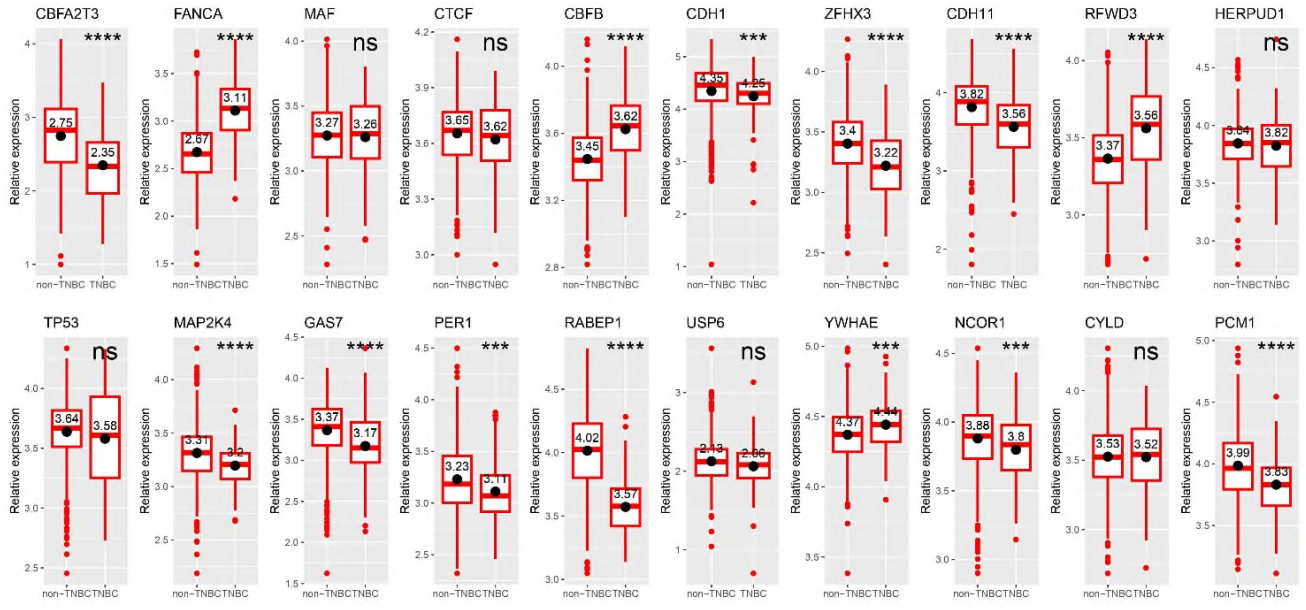

Figure 4. Comparative expression of the genes with the highest percentage of copy number loss events in breast cancer between triple-negative breast cancer (TNBC) and non-TNBC. The following convention of star symbols for statistical significance was used in the comparison of group means: ns (non-significant): $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

The Expression of BRCA2, but not of BRCA1, Is Higher in TNBC than in Non-TNBC.

Finally, the expression of the two most essential genes in the context of breast cancer (*BRCA1* and *BRCA2*) was compared between TNBC and non-TNBC samples (Figure 5). The expression of *BRCA2* was higher in TNBC than in non-TNBC (p = 2e-05) (Figure 5). However, the expression of *BRCA1* did not change between breast tumors depending on triple negativity status (TNBC vs. non-TNBC) (p = 0.16) (Figure 5).

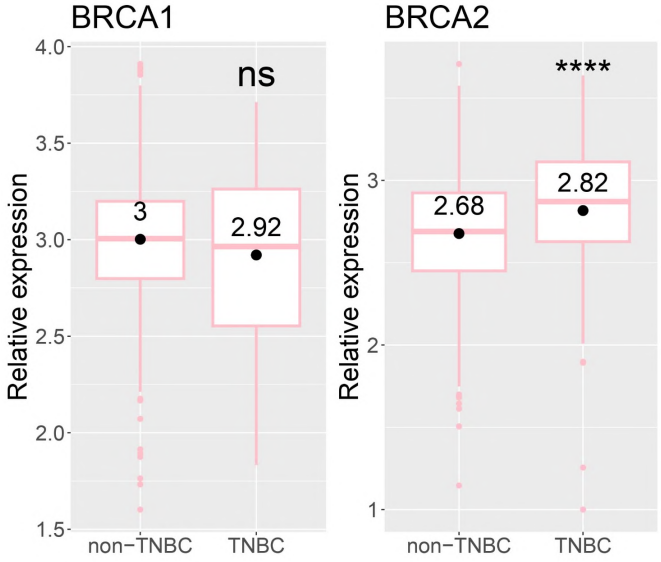

Figure 5. The expression of BRCA2, but not of BRCA1, is higher in triple-negative breast cancer (TNBC) than in non-TNBC. The following convention of star symbols for statistical significance was used in the comparison of group means: ns (non-significant): $p > 0.05$; *: $p \leq 0.05$; **: $p \leq 0.01$; ***: $p \leq 0.001$; ****: $p \leq 0.0001$.

DISCUSSION

The treatment of TNBC, the subtype with the least favorable outcome with an early tendency to metastasize to other tissues and an increased recurrence rate, remains challenging.[36,37] A better understanding of the molecular differences between TNBC and non-TNBC might contribute to the development of more targeted and molecularly guided treatment modalities with improved efficacies.

The most frequently mutated genes in patients with breast cancer are already known based on previous research; however, differential expression of these genes based on triple-negativity status has not been studied previously in a comprehensive manner. Here, 70% of the most frequently mutated genes in patients with breast cancer was first found to have decreased expression in TNBC than in non-TNBC. One of the genes whose expression was decreased in TNBC is *GATA3*. The *GATA3* functions to limit epithelial-mesenchymal transition (EMT) and metastasis in breast cancer, supporting previous observations that *GATA3* loss is associated with aggressive breast cancer development.[38,39] In more detail, it was found that the GATA3-UTX-Dicer axis can inhibit EMT, invasion, and metastasis of breast cancer cells *in vitro* and the dissemination of breast cancer *in vivo*.[38,39] Therefore, its decreased expression (or loss) in TNBC might contribute to, at least to a certain level, poor

prognosis observed in patients with TNBC by limiting the inhibition of EMT, invasion, and metastasis of breast cancer cells. One of the other genes whose expression was lower in TNBC than in non-TNBC is *PTEN*. Li et al. reported that PTEN loss might be associated with more aggressive characteristics and worse outcomes in breast cancer patients[40], again showing that decreased PTEN expression in TNBC might influence prognosis in this patient group. They found that PTEN loss is associated with larger tumor size, lymph node metastasis, high TNM stage (stage III-IV), and poor differentiation. Most importantly, their analysis showed that PTEN loss is associated with a triple-negative phenotype, supporting our findings.[40] RUNX1 transcript levels were lower in TNBC than in non-TNBC. Since RUNX1 limits aggressiveness in most subtypes of breast cancer, and RUNX1 was identified to have a role in the repression of epithelial-to-mesenchymal transition in breast cancer[41], its decreased expression in TNBC might potentially lead to increased metastatic events in this subtype of breast cancer, leading to poor prognosis. More recent studies showed that RUNX1 can repress cancer stem cells and tumorsphere formation in breast cancer.[41] Decreased RUNX1 expression in TNBC might lead to higher numbers of cancer stem cells and increased tumorsphere formation, negatively influencing prognosis in TNBC. *MUC16* expression was also observed to be higher in patients with TNBC compared with those with non-TNBC. In a very recent study, *MUC16* was shown to promote triple-negative breast cancer metastasis to the lung; thus, increased levels of MUC16 might contribute to worse outcomes in patients with TNBC.[42] Besides, although p53 is a known genetic marker for TNBCs (as the most frequently mutated gene), there was no difference in its expression between TNBC and non-TNBC at the transcript level, possibly pointing to other levels of regulation, such as protein activity or functionality or this non-significance observed can be caused due to the short half-life of p53 mRNA. Here, it also should be stated that although the difference in mean expression values for some genes between TNBC and non-TNBC is higher compared to some others, no statistically significant difference was observed as opposed to others due to the high range of distribution of expression values in each group (in TNBC and non-TNBC) for the former case.

Next, 40% of the genes with the highest percentage of copy number gain events in breast cancer showed increased expression in TNBC compared to non-TNBC. One of these genes, whose expression was increased in TNBC, is SETDB1. SETDB1, a histone methyltransferase, is known to regulate and support breast cancer metastasis.[43,44] SETDB1 is a target of miR-381-3p, whose overexpression suppresses cell proliferation, cell cycle progression, and migration in breast cancer.[44] Increased expression of SETDB1 in TNBC might contribute to disease progression into advanced tumor stages and even to endocrine therapy resistance in breast cancer.[45] Besides, the expression of MDM4 was found to be lower in TNBC compared with non-TNBC. Swetzig et al. showed that estrogen receptor alpha (ERα; ESR1) promotes the upregulation of *MDM4* in breast cancer cells, and the expression of *MDM4* is associated with ERα-positive disease[46]; therefore, decreased expression of *MDM4* in TNBC might be in part due to the absence of ERs in this subtype. Furthermore, ELF3 expression was observed to be slightly increased in TNBC. Zhang et al. showed that ELF3 is associated with a worse prognosis in patients with breast cancer.[47] Therefore, its higher levels in TNBC might influence survival negatively in patients with TNBC. Mechanistically, miR-320 (functioning as a tumor suppressor) might downregulate *ELF3* by directly binding to its 3' untranslated regions in non-TNBC cells, in addition to its function in the inhibition of the EMT and the PI3K/AKT signaling pathway in breast cancer.[47]

The half the genes with the highest percentage of copy number loss events in breast cancer was found to have decreased expression in TNBC compared to non-TNBC. The expression of *CBFA2T3* was found to be lower in TNBC. *CBFA2T3* was previously proposed as a gene with breast tumor suppressor activity.[48] ZFHX3 transcript levels were also lower in TNBC than in non-TNBC. Dong et al. reported that ZFHX3 promotes the proliferation of breast cancer cells with ER-positive status (i.e., non-TNBC), leading to tumor growth[49], possibly explaining its increased expression observed in non-TNBC. In more detail, authors showed that ZFHX3 promotes the proliferation and tumor growth of ER-positive breast cancer cells, likely by enhancing stem-like features and *MYC* and *TBX3* transcription, since they found that ZFHX3 transcriptionally activates these two genes via promoter binding.[49] mRNA levels of GAS7 were similarly shown to be decreased in TNBC. Since GAS7 was shown to reduce the number of metastatic events in particular breast cancer cells (mechanistically, GAS7 blocks CYFIP1 and Rac1 protein interaction, actin polymerization, and β1-integrin/FAK/Src signaling, leading to the suppression of breast cancer metastasis)[50], its lower levels in TNBC might conversely increase the number of metastatic events in patients with TNBC, due to the absence (or decreased activity) of this suppression axis. *CBFB* expression was found to be higher in TNBC. Hsu et al. found recently that circulating exosomes isolated from patients whose breast cancer has metastasized to the bone were rich in CBFB, and that this protein promotes more aggressive behavior in breast cancer.[51] The authors found that silencing *CBFB* in metastatic cells suppresses migration and invasion and downregulates vimentin, CXCR4, Snail1, Runx2, CD44, and OPN. Conversely, CBFB overexpression increases Runx2, vimentin, Snail1, CD44, and OPN in nonmetastatic cells.[51] Thus, it can be suggested that increased levels of *CBCF* in TNBC might influence prognosis negatively by at least promoting metastasis to the bone via the upregulation of specific genes involved in cell migration.[51] Besides, this analysis showed that *NCOR1* expression is lower in TNBC compared to non-TNBC. Since the level of *NCOR1* gene expression is an

independent prognostic factor for patients with breast cancer, and patients with high mRNA levels of *NCOR1* have a more favorable prognosis compared to those with low expression[52], its lower expression in TNBC might contribute, at least in part, to the unfavorable prognosis observed in patients with TNBC. In support, Zhang et al.[52] reported that NCOR1 mRNA is expressed at significantly higher levels in patients without axillary lymph node involvement, with a tumor size less than 2 cm, with a low or intermediate histological grade, and with ER-alpha/PR-positive and with HER2 negative tumors (i.e., non-TNBC).

Here, it should be noted that although only some of the genes studied in the present work were discussed, future work is required to better understand the functional and mechanistic details of the most of the genes covered in the context of breast cancer. Currently, studies on the most of these genes in breast cancer are highly limited. Lastly, the expression of *BRCA2*, but not of *BRCA1*, was shown to be higher in TNBC than in non-TNBC. In breast cancer patients, the tumor phenotype differs depending on the status of *BRCA1* or *BRCA2* germline mutations. Patients who carry *BRCA1* mutations mainly develop TNBC, whereas patients who carry BRCA2 mutations are more likely to have ER- and/or PR-positive breast tumors.[53-58] Therefore, it can be speculated that non-functional BRCA2 (for instance, mutant BRCA2) might be associated with ER- and/or PR-positivity in breast cancer, in parallel to the observation made in the present study that BRCA2 transcript levels are lower in breast cancer cells with ER- and/or PR-positive status. However, these inferences should be experimentally tested to make stronger assumptions.

CONCLUSION

This study provides a better understanding of the molecular differences between TNBC and non-TNBC, highlighting the need for further research to characterize the functional and clinical outcomes of these changes at the expression level between these two groups of breast cancer patients to be able to develop more personalized treatment strategies based on ER, PR and HER2 status. However, as a limitation, it should be noted that TNBC tumors also display high heterogeneity within themselves, and they can be further sub-classified based on specific driver signaling pathways, which should be taken into account when assigning TNBC patients to appropriate targeted therapies.[59,60] In other words, in addition to identifying molecular differences between TNBC and non-TNBC, determining the molecular differences within TNBC might also be of high clinical importance, considering the presence of high heterogeneity within this subtype of breast cancer.

Ethics Committee Approval: Ethics committee approval is not required for the study.

Peer Review: Externally peer-reviewed.

Conflict of Interest: Author declared no conflict of interest.

Financial Disclosure: The author is funded by the 1001 program of TUBITAK (The Scientific and Technological Research Council of Turkey) (Project no: 122S735).

ORCID IDs of the author

Caglar Berkel 0000-0003-4787-5157

REFERENCES

1. Harding C, Pompei F, Burmistrov D, Welch HG, Abebe R, Wilson R. Breast cancer screening, incidence, and mortality across US counties. *JAMA Intern Med*. 2015;175(9):1483-1489.
2. Sung H, Ferlay J, Siegel RL et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2021;71(3):209-249.
3. Kim M, Park J, Bouhaddou M, et al. A protein interaction landscape of breast cancer. *Science*. 2021;374(6563):eabf3066. doi: 10.1126/science.abf3066
4. Bianchini G, Balko JM, Mayer IA, Sanders ME, Gianni L. Triple-negative breast cancer: Challenges and opportunities of a heterogeneous disease. *Nat Rev Clin Oncol*. 2016;13(11):674-690.
5. Bianchini G, De Angelis C, Licata L, Gianni L. Treatment landscape of triple-negative breast cancer-expanded options, evolving needs. *Nat Rev Clin Oncol*. 2022;19(2):91-113.
6. Fallahpour S, Navaneelan T, De P, Borgo A. Breast cancer survival by molecular subtype: A population-based analysis of cancer registry data. *CMAJ Open.* 2017;5:E734–E739.
7. Dent R, Trudeau M, Pritchard KI, et al. Triple-negative breast cancer: Clinical features and patterns of recurrence. *Clin Cancer Res*. 2007;13:4429-4434.
8. Tan AR, Swain SM. Therapeutic strategies for triple-negative breast cancer. *Cancer J.* 2008;14:343–351.
9. Kaplan HG, Malmgren JA, Atwood M. T1N0 triple negative breast cancer: Risk of recurrence and adjuvant chemotherapy. *Breast J*. 2009;15:454–460.
10. Won KA, Spruck C. Triple negative breast cancer therapy: Current and future perspectives (Review). *Int J Oncol.* 2020;57:1245–1261.
11. Almansour NM. Triple-negative breast cancer: A brief review about epidemiology, risk factors, signaling pathways, treatment and role of artificial intelligence. *Front Mol Biosci.* 2022;9:836417. doi:10.3389/fmolb.2022.836417.
12. Berkel C, Kucuk B, Usta M, Yılmaz E, Cacan E. The effect of olaparib and bortezomib combination treatment on ovarian cancer cell lines. *Eur J Biol*. 2020;79(2):115-123.
13. Ciriello G, Gatza ML, Beck AH, et al. Comprehensive molecular portraits of ınvasive lobular breast cancer. *Cell.* 2015;163(2):506-519.
14. Jensen MA, Ferretti V, Grossman RL, Staudt LM. The NCI Genomic Data Commons as an engine for precision medicine. *Blood*. 2017;130(4):453-459.

15. Zhang Z, Hernandez K, Savage J, et al. Uniform genomic data analysis in the NCI Genomic Data Commons. *Nat Commun*. 2021;12(1):1226. doi: 10.1038/s41467-021-21254-9.
16. Berger AC, Korkut A, Kanchi RS, et al. A Comprehensive pan-cancer molecular study of gynecologic and breast cancers. *Cancer Cell*. 20189;33(4):690-705.e9. doi: 10.1016/j.ccell.2018.03.014.
17. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. *Nature*. 2012;490(7418):61-70.
18. Rahman M, Jackson LK, Johnson WE, Li DY, Bild AH, Piccolo SR. Alternative preprocessing of RNA-Sequencing data in The Cancer Genome Atlas leads to improved analysis results. *Bioinformatics*. 2015;31(22):3666-3672.
19. Weinstein JN, Collisson EA, Mills GB, et al. Cancer Genome Atlas Research Network; The Cancer Genome Atlas Pan-Cancer analysis project. *Nat Genet.* 2013;45(10):1113-1120.
20. Wilks C, Cline MS, Weiler E, et al. The Cancer Genomics Hub (CGHub): Overcoming cancer through the power of torrential data. *Database*. 2014:1–10.
21. Arora S. GSE62944: GEO accession data GSE62944 as a SummarizedExperiment. R package version 1.28.1;2023. http://bioconductor.org/packages/release/bioc/html/GSE62944.html
22. Huber W, Carey VJ, Gentleman R, et al. Orchestrating high-throughput genomic analysis with bioconductor. *Nat Methods.* 2015;12(2):115-121.
23. Gentleman RC, Carey VJ, Bates DM, et al. Bioconductor: Open software development for computational biology and bioinformatics. *Genome Biol*. 2004;5(10):R80. doi:10.1186/gb-2004-5-10-r80
24. R Core Team. R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2022. Vienna, Austria. URL https://www.R-project.org/
25. Wickham H, Averick M, Bryan J, et al. Welcome to the tidyverse. *J Open Source Softw.* 2019;4(43):1686. doi:10.21105/joss.01686
26. Wickham H. stringr: Simple, consistent wrappers for common string operations. R package version 1.5.0;2022. https://CRAN.R-project.org/package=stringr
27. Wickham H. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New York, 2016.
28. Wickham H, Bryan J. readxl: Read Excel Files. R package version 1.4.2;2023. https://CRAN.R-project.org/package=readxl
29. Morgan M, Shepherd L. ExperimentHub: Client to access ExperimentHub resources. R package version 2.4.0;2022.
30. Morgan M, Obenchain V, Hester J, Pagès H. SummarizedExperiment: SummarizedExperiment container. R package version 1.26.1;2022. https://bioconductor.org/packages/SummarizedExperiment
31. Kassambara A. ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.6.0;2023. https://CRAN.R-project.org/package=ggpubr
32. Allaire J, Xie Y, Dervieux C, et al. rmarkdown: Dynamic Documents for R. R package version 2.21;2023. https://github.com/rstudio/rmarkdown
33. Xie Y. knitr: A General-Purpose Package for Dynamic Report Generation in R. R package version 1.42;2023.
34. Berkel C, Cacan E. *In silico* analysis of DYNLL1 expression in ovarian cancer chemoresistance. *Cell Biol Int*. 2020;44(8):1598-1605.
35. Berkel C, Cacan E. Transcriptomic analysis reveals tumor stage-or grade-dependent expression of miRNAs in serous ovarian cancer. *Hum Cell*. 2021;34(3):862-877.
36. Li X, Yang J, Peng L, et al. Triple-negative breast cancer has worse overall survival and cause-specific survival than non-triple-negative breast cancer. *Breast Cancer Res Treat.* 2017;161(2):279-287.
37. Bai X, Ni J, Beretov J, Graham P, Li Y. Triple-negative breast cancer therapeutic resistance: Where is the Achilles' heel? *Cancer Lett.* 2021;497:100-111.
38. Bai F, Zhang LH, Liu X, et al. GATA3 functions downstream of BRCA1 to suppress EMT in breast cancer. *Theranostics*. 2021;11(17):8218-8233.
39. Yu W, Huang W, Yang Y, et al. GATA3 recruits UTX for gene transcriptional activation to suppress metastasis of breast cancer. *Cell Death Dis*. 2019;10(11):832. doi:10.1038/s41419-019-2062-7
40. Li S, Shen Y, Wang M, et al. Loss of PTEN expression in breast cancer: Association with clinicopathological characteristics and prognosis. *Oncotarget*. 2017;8(19):32043-32054.
41. Hong D, Fritz AJ, Gordon JA, et al. RUNX1-dependent mechanisms in biological control and dysregulation in cancer. *J Cell Physiol*. 2019;234(6):8597-8609.
42. Chaudhary S, Appadurai MI, Maurya SK, et al. MUC16 promotes triple-negative breast cancer lung metastasis by modulating RNA-binding protein ELAVL1/HUR. *Breast Cancer Res*. 2023;25(1):25. doi:10.1186/s13058-023-01630-7
43. Ryu TY, Kim K, Kim SK, et al. SETDB1 regulates SMAD7 expression for breast cancer metastasis. *BMB Rep*. 2019;52(2):139-144.
44. Wu M, Fan B, Guo Q, et al. Knockdown of SETDB1 inhibits breast cancer progression by miR-381-3p-related regulation. *Biol Res*. 2018;51(1):39. doi: 10.1186/s40659-018-0189-0
45. Liu Z, Liu J, Ebrahimi B, et al. SETDB1 interactions with PELP1 contributes to breast cancer endocrine therapy resistance. *Breast Cancer Res.* 2022;24(1):26. doi: 10.1186/s13058-022-01520-4
46. Swetzig WM, Wang J, Das GM. Estrogen receptor alpha (ERα/ESR1) mediates the p53-independent overexpression of MDM4/MDMX and MDM2 in human breast cancer. *Oncotarget*. 2016;7(13):16049-16069.
47. Zhang Z, Zhang J, Li J, et al. miR-320/ELF3 axis inhibits the progression of breast cancer via the PI3K/AKT pathway. *Oncol Lett*. 2020;19(4):3239-3248.
48. Kochetkova M, McKenzie OL, Bais AJ, et al. CBFA2T3 (MTG16) is a putative breast tumor suppressor gene from the breast cancer loss of heterozygosity region at 16q24.3. *Cancer Res*. 2002;62(16):4599-4604.
49. Dong G, Ma G, Wu R, et al. ZFHX3 promotes the proliferation and tumor growth of ER-positive breast cancer cells likely by enhancing stem-like features and MYC and TBX3 transcription. *Cancers (Basel)*. 2020;12(11):3415. doi: 10.3390/cancers12113415
50. Chang JW, Kuo WH, Lin CM, et al. Wild-type p53 upregulates an early onset breast cancer-associated gene GAS7 to suppress metastasis via GAS7-CYFIP1-mediated signaling pathway. *Oncogene*. 2018;37(30):4137-4150.
51. Hsu CH, Ma HP, Ong JR, et al. Cancer-associated exosomal CBFB facilitates the aggressive phenotype, evasion of oxidative stress, and preferential predisposition to bone prometastatic factor of breast cancer progression. *Dis Markers*. 2022;2022:8446629. doi: 10.1155/2022/8446629
52. Zhang Z, Yamashita H, Toyama T, et al. NCOR1 mRNA is an independent prognostic factor for breast cancer. *Cancer Lett*. 2006;237(1):123-129.
53. Mavaddat N, Barrowdale D, Andrulis IL, et al. Consortium

of Investigators of Modifiers of BRCA1/2. Pathology of breast and ovarian cancers among BRCA1 and BRCA2 mutation carriers: results from the Consortium of Investigators of Modifiers of BRCA1/2 (CIMBA). *Cancer Epidemiol Biomarkers Prev*. 2012;21(1):134-147.

54. De Talhouet S, Peron J, Vuilleumier A, et al. Clinical outcome of breast cancer in carriers of BRCA1 and BRCA2 mutations according to molecular subtypes. *Sci Rep*. 202027;10(1):7073. doi:10.1038/s41598-020-63759-1 Erratum in: *Sci Rep*. 2020;10(1):19248.
55. Lakhani SR, Reis-Filho JS, Fulford L, et al. Prediction of BRCA1 status in patients with breast cancer using estrogen receptor and basal phenotype. *Clin Cancer Res*. 2005;11:5175–5180.
56. Armes JE, Trute L, White D, et al. Distinct molecular pathogeneses of early-onset breast cancers in BRCA1 and BRCA2 mutation carriers: a population-based study. *Cancer Res.* 1999;59:2011–2017.
57. Palacios J, Honrado E, Osorio A, et al. Phenotypic characterization of BRCA1 and BRCA2 tumors based in a tissue microarray study with 37 immunohistochemical markers. *Breast Cancer Res Treat.* 2005;90:5–14.
58. Lakhani SR, Van De Vijver MJ, Jacquemier J, et al. The pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. *J Clin Oncol*. 2002;20:2310–2318.
59. Lehmann BD, Bauer JA, Chen X, et al. Identification of human triple-negative breast cancer subtypes and preclinical models for selection of targeted therapies. *J Clin Invest*. 2011;121(7):2750-2767.
60. Sporikova Z, Koudelakova V, Trojanec R, Hajduch M. Genetic markers in triple-negative breast cancer. *Clin Breast Cancer.* 2018;18(5):e841-e850. doi: 10.1016/j.clbc.2018.07.023

How to cite this article

Berkel C. Retrospective Analysis of Transcriptomic Differences between Triple-Negative Breast Cancer (TNBC) and non-TNBC. Eur J Biol 2024; 83(1): 19–27. DOI:10.26650/EurJBiol.2024.1362117