Original Article

Eur J Breast Health 2024; 20(3): 178-184
DOI: 10.4274/ejbh.galenos.2024.2024-3-7

Bioinformatic Investigation of Genetic Changes in Paraoxonase Genes in Breast Cancer and Breast Cancer Subtypes

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ABSTRACT

Objective: Among women, breast cancer (BC) is the most prevalent form of cancer. Many molecular targets have been discovered for BC prognosis and treatment. However, new markers still need to be identified, as cancer pathogenesis is triggered by different mechanisms. The aim of this study was to examine the changes in the *paraoxonase* genes (*PON1*, *PON2*, and *PON 3*) involved in the pathogenesis of BC.

Materials and Methods: The characteristics of the mutations were evaluated with the Cbioportal database. Kaplan-Meier Plot evaluated recurrence-free survival (RFS). The UALCAN database determined the promoter methylation. Gene expression was evaluated by GEPIA2.0 database.

Results: PON1 harbored the most mutations. There was a significant decrease in PON3 expression levels in BC samples compared with healthy samples. PON1 and PON2 expression levels did not differ between BC tissue and normal adjacent tissue. Elevated expression levels of *PON1* and *PON2* genes were correlated with longer RFS, whereas reduced expression of the *PON2* gene showed an association with longer RFS. Moreover, the promoter regions of PON1 and PON 3 were found to be hypermethylated, while the promoter region of PON 2 was found to be hypomethylated. The PON3 promoter region was significantly hypermethylated in luminal and human epidermal growth factor receptor 2 (HER2) + BC subtypes. However, the PON3 promoter region was significantly hypomethylated in the triple negative breast cancer (TNBC) subtype.

Conclusion: These results suggest that methylation and expression status of PON3 in BC and BC subtypes (TNBC, luminal and HER2) may indicate a poor prognosis. The *PON3* gene could be a negative prognostic marker in BC. However, the results should be supported by prospective studies.

Keywords: Breast cancer; paraoxonase; bioinformatics

Cite this article as: Ayan D, Gül MA, Karabay U, Bulut SM. Bioinformatic Investigation of Genetic Changes in Paraoxonase Genes in Breast Cancer and Breast Cancer Subtypes.Eur J Breast Health 2024; 20(3): 178-184

Key Points

- Breast cancer is one of the most common cancer types among women.
- Paraoxonase 1 (PON1) enzyme has the most mutations compared to PON2 and PON3 enzymes.
- *PON3* gene expression level (downregulation) in breast tissue was significant ($p<0.05$)
- PON3 expression levels were downregulated, especially in triple negative breast cancer (TNBC)-BL1, TNBC-BL2 and TNBC-unknown subtypes
- The PON3 promoter region was significantly hypomethylated in the TNBC subtype. This may explain global DNA hypomethylation.
- Global DNA hypomethylation in breast cancer may be associated with the formation of repressive chromatin domains and gene silencing

Introduction

Breast cancer (BC) is one of the most common cancers in women (1, 2). The high mortality and morbidity rates associated with BC make it a prominent health concern among women. Despite adjuvant chemotherapy, the five-year survival rate for metastatic BC is less than 30% (3). Although the precise cause of BC is not yet fully understood, specific risk factors can heighten the probability of its occurrence. These risk factors include age (the risk increases with age), a family history of BC and genetic mutations (e.g. *BRCA1* and *BRCA2*) (4). There are five different subtypes of BC, known as luminal A [estrogen receptor (ER), progesterone receptor (PR)+, human epidermal growth factor receptor 2 (HER2)–, Ki67+ <20%], luminal B (ER/PR+ <20%, HER2–, Ki67+ ≥20%) HER2+ B2 (ER/PR+, HER2 overexpression), HER2 overexpression (ER–, PR–, HER2 overexpression) and triple

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Received: 18.03.2024
Accepted: 27.03.2024
Available Online Date: 01.07.2024

negative breast cancer-basal-like (TNBC-BL) (ER–, PR–, HER2–). The most challenging of these is TNBC, where all receptors are negative, treatment options are limited, and it has an aggressive course. For this reason, the search for new treatments for this type of cancer continues (5).

The *paraoxonase (PON)* gene is located on human chromosome 7 (6). The enzyme PON is involved in antioxidant defense and is associated with lipid metabolism. There are three isoforms within the enzyme class, namely PON1, PON2 and PON3. PON1, which is mainly found in the bloodstream, is known for its ability to hydrolyse and detoxify specific harmful compounds, such as organophosphates. In addition, PON1 has an antioxidant effect and protects cells from oxidative stress caused by reactive oxygen species (ROS) (7). The antioxidant capacity of PON1 suggests that it may play a protective role against cancer development by neutralizing harmful ROS that can damage DNA and promote tumour formation.

Several studies have explored the relationship between PON and the risk and prognosis of BC, although the precise mechanisms are not yet fully understood (8). Some evidence suggests that PON1 activity may be associated with treatment outcomes in BC patients (9). Human susceptibility to BC has been studied in the context of genetic variations in the PON. Some specific single nucleotide polymorphisms in the *PON1* gene have been associated with altered enzyme activity that may affect a person's risk of developing BC (8). It has also been reported that higher levels of PON1 in BC tissue than in normal tissue indicate a possible link to cancer development (10). PON1 and PON3, which are bound to high-density lipoprotein (HDL) particles, have antioxidant and anti-inflammatory properties (11). PON2 and PON3 serve as intracellular enzymes and regulate mitochondrial superoxide anion production and endoplasmic reticulum (ER) stress-induced apoptosis (12). The pleiotropic role of PON enzymes has been studied in the context of cardiovascular and neurodegenerative diseases (13). In recent decades, researchers have identified the overexpression of PON2 and PON3 in cancer cells, leading to the suggestion that these enzymes may contribute to tumour survival and resistance to stress (11).

However, the clinical utility of PON enzymes as diagnostic or prognostic biomarkers remains unclear and requires further validation. Given the current state of knowledge, in this study we used some bioinformatics tools to analyze the association among possible mutations, expression and promotor methylation of *PON1*, *PON2* and *PON3* genes in BC subtypes, especially TNBC.

Material and Methods

Study Design

This was a bioinformatics study. The current study was based upon open-source data obtained from The Cancer Genome Atlas (TCGA, https://www.cancer.gov/tcga), which is a public database. The patients involved in the database have given ethical approval. Users can download relevant data for free for research and publish relevant articles. There are no ethical issues or other conflicts of interest. The BRCA cohort consists of a total of 996 cases. The results of these cases were obtained using the Cbioportal database. The data were accessed on July 05, 2023, from TCGA. The cbioportal database evaluated PON1, PON2, and PON3 mutations. This database accessed the amino acid in which the mutation occurred, the cancer subtype, and clinical information about the cancer. It was determined whether there was a somatic mutation or not, using the COSMIC (https://cancer.sanger.ac.uk/cosmic) database.

Survival Investigation

For recurrence-free survival (RFS) analysis, the Kaplan-Meier Plotter (KM) tool, available at https://kmplot.com/analysis/, was used to analyze the associations between gene expressions and survival rates in cancer. Moreover, this tool helped us to understand the prognostic values of the expression levels of *PON1*, *PON2* and *PON3* genes in BC patients. The KM plotter can evaluate the correlation between the expression of PON1, PON2 and PON3 (mRNA) and the survival rate in BC. The KM-Plotter used Cox proportional hazards regression and calculates the false discovery rate to analyze and evaluate the data (14).

Gene Expression and Correlation

We used Gene Expression Profiling Interactive Analysis, version 2 (GEPIA2.0) to assess the expression levels of PON1, PON2 and PON3 in both tumour and adjacent normal tissues, focusing specifically on BC. GEPIA2.0 uses the TCGA database and genotype-tissue expression (GTEx) data to perform this analysis. The screening criteria used in GEPIA2.0 were $p<0.05$, and |Log2FC| the cut-off point was 0.01. These criteria filter out genes that are not significantly differentially expressed between the two datasets (15). In addition, the correlation analysis between the expressions of the genes was examined with the GEPIA2.0 database.

Promoter Methylation and BC Subtypes Expression

UALCAN is an interactive open-access web page for OMICS data analysis (http://ualcan.Path.uab.edu/index.html). This database is built on PERL-CGI and can be used at approximately 6000 gene methylation levels (16). This study evaluated the promoter methylation level of *PON1*, *PON2*, and *PON3* genes in the BRCA data. Promoter region methylation levels of genes were also examined in BC subtypes. In addition, the change in expression levels in BC patients with BC subtypes accompanied by the TNBC subtype was examined using the UALCAN web server, since expression analysis was not available in the other web tool (GEPIA2).

Statistical Analysis

The evaluation of the study data included statistical analyses performed with the GEPIA2 database. Differential expression between BC tissue and adjacent normal tissue was measured using the One-Way ANOVA test. Correlation analysis of genes was performed by Pearson correlation analysis. The post-hoc Tukey test was favored for homogeneity of variances. Significance of difference between methylation levels was estimated by Student's t-test in UALCAN database. Significance of difference between BC subtypes was evaluated by the One-Way ANOVA test in UALCAN database. Survival status was assessed using the KM plotter with default settings, focusing on recurrence-free survival and using auto-best cut-off values and the J-best probe set. A log-rank p-value below 0.05 was considered statistically significant.

Results

Mutation Profile Interpretation

Among 996 cases, 9 cases (0.9%) of BC patients had genetic alterations in PON1, PON2 and PON3. There was a statistical significant co-occurrence of mutations in the analyzed populationamong PON1, PON2, and PON3 ($p<0.01$). The highest mutation rates were found in PON1 (55.5%), followed by PON3 (33.3%) and PON2 (11.2%).

The mutation types in the *PON1*, *PON2* and *PON3* genes in BC are listed in Table 1. In the analyzed genes of the BRCA cohort, there were eight missense mutations (88.8%) and one nonsense mutation (11.2%). None of these mutations originated in germ cells. The COSMIC database confirmed the somatic origin of the mutations.

The *PON1* gene contains two evolutionarily conserved regions. One is the arylesterase (between 168-253 amino acids) and the other is the SMP-30/gluconolaconase/LRE-like region (92-295 amino acids). There are three missense mutations in the region where the arylesterase domain of PON1 is located. All three of these mutations are located in the exon 7 region. In addition, there was a nonsense mutation (exon 4 region) and another missense mutation (exon 8 region). PON2 contains a 100% conserved arylesterase domain in the evolutionary process. This domain is located between 99 and 184 amino acid residues. There was a missense mutation in PON2 outside this region. This mutation was located in the exon 5 region. PON3 also contains an evolutionarily conserved arylesterase domain. This region is located between 167-252 amino acid residues. There was a missense mutation in this region of PON3, located in the exon 6 region. In addition, there were two further missense mutations in this gene. These mutations are located in the regions of exon-9 and exon-8.

Survival Prognosis Evaluation Results of Genes

The RFS results we obtained from the KM plotter analysis are shown in Figure 1. According to the analysis results, we found that increased expression of the *PON1* and *PON3* genes was significantly related to longer RFS (p = 7.5E-06 and p = 3.3E-05, respectively), while decreased expression of the *PON2* gene was significantly related to longer RFS (p = 0.024).

Gene Expression Results

The expression level of the *PON3* gene in tumour tissue was significantly lower when comparing tumour tissue (n = 1085) with adjacent normal tissue (n = 291) (p = 0.01), but there was no difference in the expression levels of the *PON1* and *PON2* genes (according to GEPIA2) (Figure 2A).

According to UALCAN, the expression level of PON1 in BRCA, based on major subclasses (with TNBC types) was significantly downregulated in TNBC Basal-like 1 (TNBC-BL1) (n = 13) compared to adjacent normal tissue (n = 114) (p<0.001). The expression level of PON2 in BRCA based on major subclasses (with TNBC types) was significantly upregulated in Luminal (n = 566) and downregulated in HER2+ (n = 37) compared to adjacent normal tissue (n = 114) (p<0.001). The expression level of PON3 in BRCA based on major subclasses (with TNBC types) was significantly downregulated in TNBC-BL1 (n = 13), TNBC Basal-like 2 (TNBC-BL2) (n = 11) and TNBC unspecified (TNBC-UNS) (n = 27) compared to adjacent normal tissue (n = 114) (p<0.001) (Figure 2B).

While there was a weak positive correlation between *PON1* gene expression levels and *PON2* gene expression levels (r = 0.18, p<0.001), a strong positive correlation was found between *PON1* gene expression levels and *PON3* gene expression levels (r = 0.095, p = 0.00041). In addition, there was a weak positive correlation between *PON2* gene expression levels and *PON3* gene expression levels (r = 0.28, p<0.001) (Figure 3).

Analyzes of Promoter Methylation Level

DNA methylation is an essential condition for the epigenetic modification of the genome and is closely linked to the development of cancer (17). According to the results of the analysis using the UALCAN online tool to determine the DNA methylation level, the promoter methylation level of PON1 (p = 1.62E-12) and PON3 (p = 1.62E-12) was found to be significantly higher (hypermethylation) in BC tissue compared to healthy tissue. Conversely, the methylation level of the promoter of PON2 (p = 3.79E-03) was significantly lower (hypomethylation) (Figure 4A). Moreover, compared to normal tissue, the PON3 promoter region methylation level was hypermethylated

Table 1. *PON1*, *PON2* and *PON3* gene mutation analysis

No	Gene	Nt change	AA position	Type of cancer	Subtype	American Joint Committee on Cancer Metastasis Stage Code	Neoplasm Disease Lymph Node Stage American Joint Committee on Cancer Code	Neoplasm Disease Stage American Joint Committee on Cancer Code	Diagnosed age	Overall survival (months)
1	PON1	c.743A>G	***Y248C***	BIDC	Basal like	M0	N0	STAGE IIA	69	16.4*
2	PON1	c.736C>T	***H246Y***	BIDC	Luminal B	M0	N0	STAGE I	60	13.0*
3	PON1	c.821A>T	***D274V***	BIDC	Basal like	M0	N1	STAGE IIA	40	36.3*
4	PON1	c.332C>A	***S111****	BIDC	Luminal B	M0	N1A	STAGE IIB	77	14.1*
5	PON1	c.727C>T	***H243Y***	BIDC	Basal like	MX	N1A	STAGE IIB	49	14.8*
6	PON2	c.396C>A	***N132K***	BIDC	Luminal B	M0	N0 (I-)	STAGE IIA	69	81.6
7	PON3	c.683C>T	***S222L***	BIDC	Basal like	M1	N2	STAGE IV	59	7.9*
8	PON3	c.929T>G	***L310W***	BILC	HER2+	MX	N0	STAGE IIA	65	16.6*
9	PON3	c.882C>G	***N294K***	BILC	Luminal A	M0	N2A	STAGE IIIA	72	47.0

BIDC: Breast ınvasive ductal carcinoma; BILC: Breast ınvasive lobular carcinoma; *: Livin; HER2: Human epidermal growth factor receptor 2

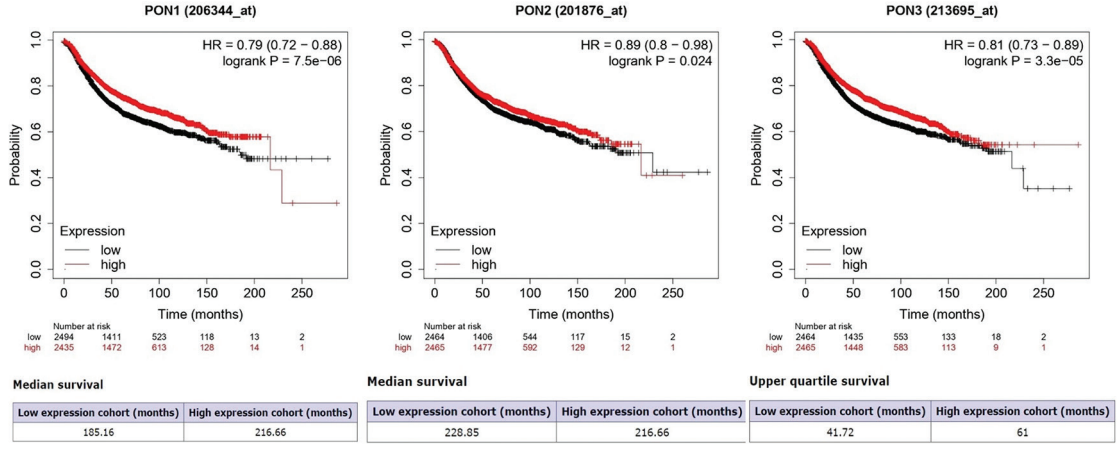

Figure 1. Different expressions of PON1, PON2 and PON3 in breast cancer (BC) patients in the recurrence-free survival curve (using the Kaplan-Meier plotter). The red line represents the survival rate curve of patients with BC who expressed the gene, and the black line represents the survival rate curve of BC patients who did not express the gene

in the luminal and HER2 positive BC subtypes (p = 1.62E-12, p = 8.83E-04, respectively), while it was hypomethylated in the TNBC (p = 3.39E-03). In BC subtypes accompanied by TNBC, PON3 expression levels were downregulated compared to normal tissue. However, significance was present only for TNBC-BCL1, TNBC-BCL1, TNBC-UNS (p = 5.44E-03, p = 1.40E-03 and p = 7.58E-03, respectively) (Figure 4B).

Discussion and Conclusion

As a multifactorial antioxidant enzyme, PON1 plays a protective role against oxidative stress, a factor thought to contribute to the development of cancer. The role of PON1 in the detoxification of cancer-causing oxidative stress has led to the evaluation of genetic variations in PON1, particularly with regard to susceptibility to BC (18). Considering the data obtained through the cBioPortal, five missense mutations in the *PON1* gene were discovered. PON1 is the gene in the paraoxonase gene family that underwent the most somatic alterations. The p.S111* nonsense changes in the exon 4 region may cause the polypeptide to terminate too early and the protein to be truncated. This probably leads to the loss of the arylesterase domain. The presence of an arylesterase domain characterizes the PON family of genes. Arylesterase enzymes, which belong to the family of conserved protein domains, are responsible for the hydrolysis of organophosphorus esters such as paraoxon. These enzymes are present both in the liver and in the blood and ensure resistance to the toxicity of organophosphates. In humans, arylesterase is associated with HDL and presumably provides protection against oxidation of low-density lipoprotein (19). There were three different (p. H243Y, p. H246Y, p.Y248C) missense mutations in this 100% conserved splice region. The result of these is likely to be dysfunctional protein. In this case, the antioxidant balance, which has an important role in the pathogenesis and development of BC, may be disrupted.

Hypermethylation in the PON1 promoter region has been reported to lead to downregulation of PON1 (20). In the BRCA cohort, the promoter region of PON1 in BC tissue was statistically hypermethylated compared to healthy tissue. In our BRCA cohort, the expression of PON1 was decreased in BC tissue compared to healthy tissue, possibly as a result of the hypermethylation of the promoter region. When we examined BC subtypes, we found that luminal and HER2+ were hypermethylated compared to normal tissue, but on the other hand, the TNBC subtype was hypomethylated. When it comes to expression levels, we found significant downregulation only in TNBC-BL1. Although this does not conform with the classical theory, it can best be explained by global DNA hypomethylation. Global DNA hypomethylation is a hallmark of human cancer, but its functional consequences remain unclear. The results of the present study suggest that global DNA hypomethylation in BC is closely linked to the formation of repressive chromatin domains and gene silencing. Therefore, this can be described as a potential epigenetic pathway for gene regulation in cancer cells (21).

Reduced PON1 expression was associated with shorter RFS in BC patients. Tumor growth and development depend on a wide variety of factors. The most prominent of these are inflammation and oxidative stress. The inflammatory and oxidative environment contributes to cell damage and may lead to cancer formation in the long-term (22). However, differential DNA methylation, detection of circulating nucleic acids, and histone-modifying enzymes are being investigated as epigenetic biomarkers for BC (21). In light of this information, an inflammatory and oxidative environment may occur as a result of the decrease in PON1 expression levels in the BRCA cohort due to the effect of epigenetic mechanisms and may cause serious prognostic effects by supporting the progression of cancer pathogenesis in patients in the long-term.

In the BRCA cohort, there was a missense mutation in the *PON2* gene. The *PON2* gene was the least somatically mutated of the *PON* genes analyzed. The promoter region of PON2 was significantly hypomethylated in BC tissue. This may explain the upregulation of PON2 in the BRCA cohort. On the other hand, the promoter region of PON2 contains an ER stress element-like sequence that regulates ER stress (6). Epigenetic changes in this region can cause ER stress in particular. There is evidence that the enzyme PON2 plays a role in cancer cell survival due to its antioxidant and anti-apoptotic activities. In addition, it is thought to play a role in the resistance of cancer cells to chemotherapy and therefore RFS (11). Thus, the upregulation of PON2 in BC tissue may be considered a survival mechanism of cancer cells. It has been observed that PON2 levels are upregulated in patients with BC (23). Although the expression of PON2 tended to

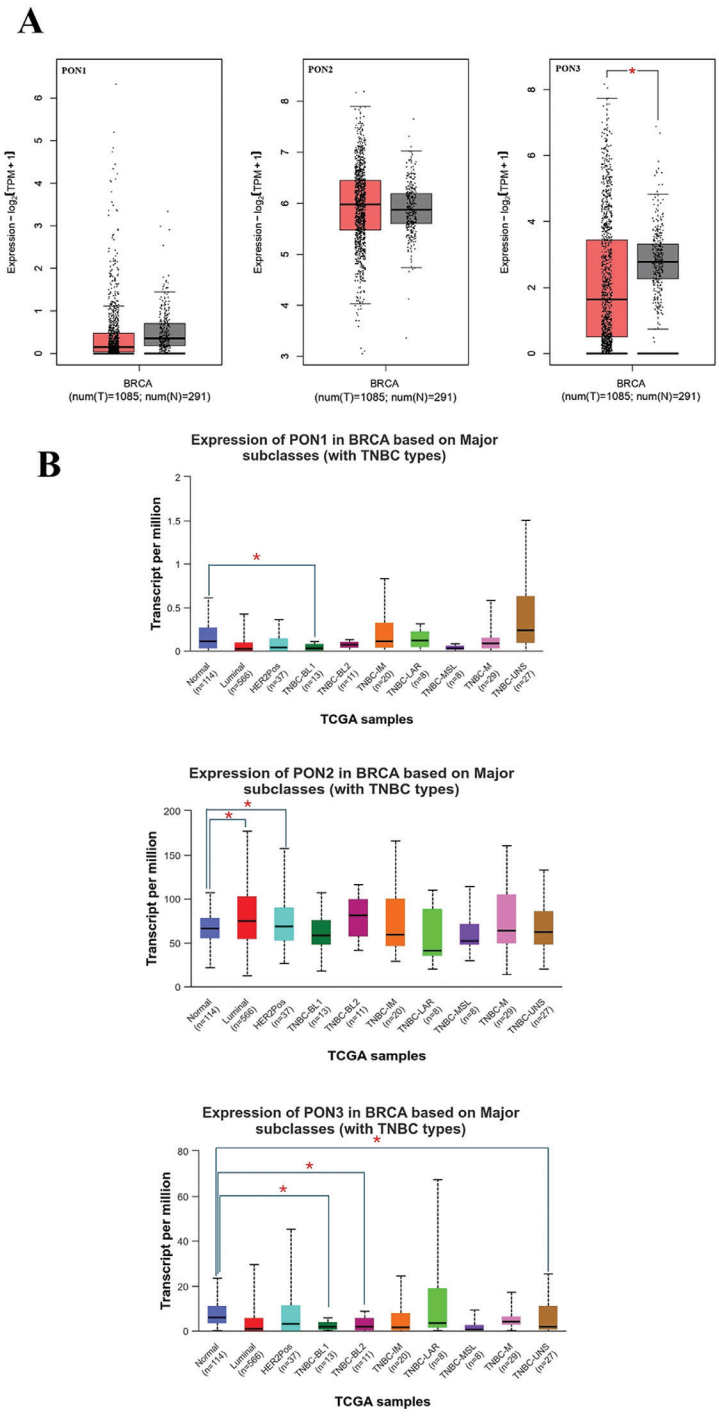

Figure 2. A. mRNA expressions of PON1, PON2 and PON3 in BC (red) and normal breast tissues (gray). **B.** The expression level of PON1, PON2 and PON3 in BRCA based on major subclasses (with TNBC types), *: $p<0.05$

TNBC-BL1: TNBC basal-like 1; TNBC-BL2: TNBC basal-like 2; TNBC-IM: TNBC immunomodulatory; TNBC-LAR: TNBC luminal androgen receptor; TNBC-MSL: TNBC mesenchymal stem-like; TNBC-M: TNBC mesenchymal; TNBC-UNS: TNBC unspecified; BC: Breast cancer; TNBC: Triple negative breast cancer

be upregulated in the BRCA cohort compared to expression in healthy tissue, there was no significant difference between these two tissues. Interestingly, higher PON2 expression levels were associated with shorter RFS in the BRCA cohort. Although this situation seems to be associated with poor prognosis in BC, the fact that the increase in PON2 levels was not significant in the BRCA cohort makes it difficult to evaluate it from a prognostic perspective.

In a study of infiltrating tumours related to BC subtypes, such as luminal A, HER2+ and TNBC, PON2 expression was significantly upregulated compared to expression levels in healthy tissue (23). TNBC is highly aggressive, prone to recurrence and early metastasis, and lacks receptors for targeted therapy, limiting treatment options to surgery, radiotherapy, and chemotherapy. It has been observed that PON2 levels are overexpressed in this BC subtype, and it has been proposed that reducing PON2 levels may be a treatment option. As a result, it was found that when PON2 expression levels were reduced, BC cell proliferation decreased and the response to chemotherapy was more effective in TNBC cells (23). In the BRCA cohort, while there was statistical difference in the PON2 expression levels of tumour tissue compare to adjacent normal tissue in luminal and HER2+ subtypes, there was no any statistically difference TNBC subtype. Furthermore, there was only one mutation in the *PON2* gene in the BRCA cohort, and the BC subtype associated with this mutation was luminal B. Therefore, more evidence is needed to consider PON2 as a treatment option in subtypes of BC.

Three missense mutations were found in the *PON3* gene in our BRCA cohort. One (p.S228L missense mutation) is a 100% conserved splice site in the arylesterase domain. Such a change in the arylesterase domain may affect the progression of cancer pathogenesis by causing a change in the antioxidant property of the protein. As with other PON family genes, increased expression levels in PON3 seem to be associated with prolonged survival.

PON3 is overexpressed in a proportion of various human tumors, just like PON2, and reduces mitochondrial superoxide formation (11). Direct interaction with coenzyme Q10 characterizes PON3, suggesting its probable function in sequestering ubisemiquinone and enhancing resistance to cell death. PON3 is situated within the ER and mitochondria, and it counteracts apoptosis triggered by DNA damage or intrinsic and extrinsic stimuli. In addition, PON3 impairs ER stress-induced apoptotic MAPK signaling and CHOP induction. In this case, the upregulation of PON3 in cancer tissue protects the cell against mitochondrial superoxide-mediated cell death (11). Studies have obtained various and different data on the regulation of PON3 in cancer tissue. One indicated that PON3 was underexpressed in 13 out of 20 cancer types, including bladder, breast, colorectal, liver, and lung cancer (24). Other studies reported that PON3 level was high in cancers such as pancreas, lung, prostate, and liver (25). In the BRCA cohort, the expression levels of the *PON3* gene in breast tissue were significantly lower when compared to healthy tissue. This decrease can be attributed to the hypermethylation of the *PON3* gene promoter region in the BRCA cohort. In addition, lower PON3 expression levels were identified to be associated with shorter RFS in the BRCA cohort. Furthermore, according to GEPIA2, tumor tissue PON3 expression levels were significantly downregulated compared to adjacent normal tissue across BC subtypes (except for the Basal Like subtype). We found very interesting results when evaluating the PON3 promoter region methylation status in BC subtypes. The PON3 promoter region was significantly hypermethylated in luminal and HER2 positive BC subtypes, and PON3 expression levels were significantly downregulated in both luminal and HER2 positive BC subtypes. This suggests gene silencing because of promoter region hypermethylation. In contrast, in our BRCA cohort, the PON3 promoter region was significantly

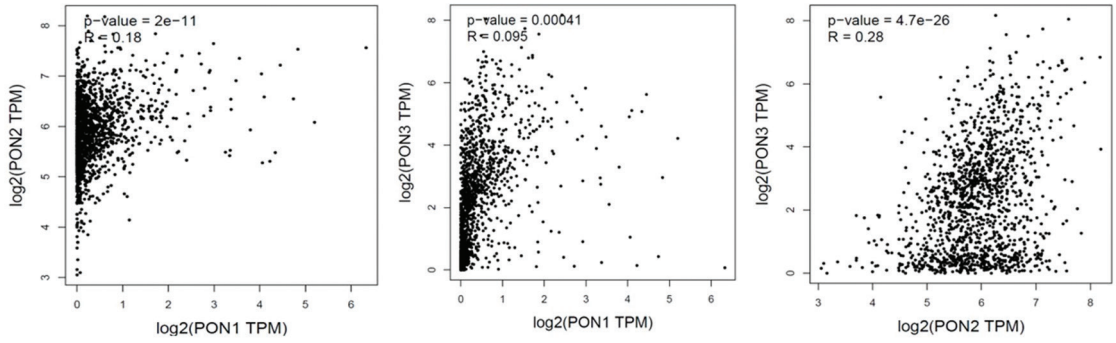

Figure 3. The correlation results of PON1, PON2 and PON3

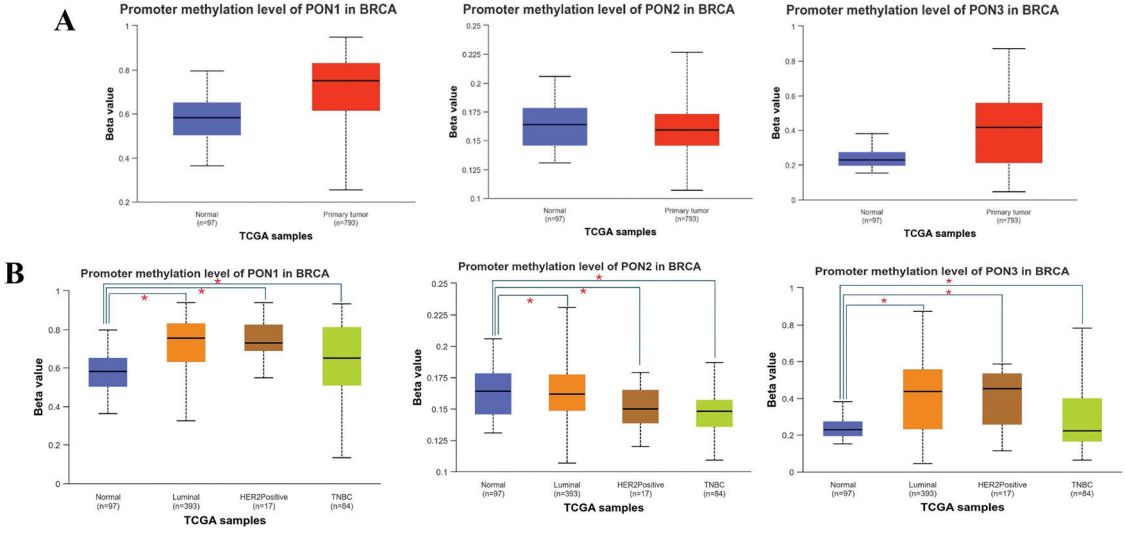

Figure 4. A. The promoter methylation level of PON1, PON2 and PON3. **B.** The promoter methylation level of *PON1*, *PON2* and *PON3* genes according to luminal, HER2+, and TNBC, *: $p<0.05$

TNBC: Triple negative breast cancer; HER2: Human epidermal growth factor receptor 2

hypomethylated in the TNBC subtype. According to classical theory, it is expected that the gene will be activated and its expression levels will increase as a result of promoter region hypomethylation (26). However, PON3 expression levels were downregulated, especially in TNBC-BL1, TNBC-BL2 and TNBC-unknown subtypes. This may again be a result of global DNA methylation and global DNA hypomethylation in BC could be associated with the formation of repressive chromatin domains and gene silencing.

Especially in TNBC, this potential epigenetic change should be evaluated in further research in terms of regulating the *PON* genes. This may help identify new pathogenetic mechanisms and treatment options. However, we believe that further, population-based studies are needed to elucidate this epigenetic pathway, which remains unclear.

Study Limitations

Although our study was well designed, there are some limitations. The study includes the results of a study conducted in a limited area of population. For this reason, priority should be given to studies to be carried out with wider participation in different populations. Additionally, since the results are obtained through the bioportal, not all data regarding the cases may be available.

Our findings suggest that, in line with the data obtained in the BRCA cohort, changes in the *PON3* gene in BC and BC subtypes appear to be a genetic risk factor for BC development and can be considered an applicable molecular biomarker for screening women with a genetic predisposition. The current study may contribute to the literature to understand the possible impact of changes in the molecular function of genes belonging to the PON family on the pathogenesis of BC. As a result of the data obtained, the methylation status of PON3 in BC and BC subtypes, especially TNBC, may indicate a poor prognosis. This data may suggest a search for new therapeutic strategies targeting PON3. However, we suggest prospective investigations with BC driver gene mutations may be required to appraise *PON* genes as a treatment target strategy. Since the mechanisms behind cancer are many, there is a need for large-scale research on this subject by adopting population-based and multidisciplinary approaches.

Ethics Committee Approval: The data used in our study were obtained from public database TCGA, therefore, ethical approval was not required.

Informed Consent: Not necessary.

Authorship Contributions

Surgical and Medical Practices: D.A., S.M.B.; Concept: D.A., M.A.G.; Design: D.A., M.A.G., U.K.; Data Collection and/or Processing: D.A., U.K., S.M.B.; Analysis and/or Interpretation: D.A., M.A.G.; Literature Search: D.A., U.K.; Writing: D.A., S.M.B.

Conflict of Interest: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

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