

ORIGINAL RESEARCH ARTICLE

Eur J Med Transl Biosci. 2026;1(1):20–25 · Open Access · CC BY 4.0 · Selected from ICMTB, peer reviewed by members of the editorial board

INTEGRATIVE ANALYSIS OF GENETIC VARIATION IN BREAST CANCER: A RETROSPECTIVE STUDY OF THE ALBANIAN COHORT

Merita Xhetani^{1,2,3*}, Albina Hasa⁴

¹*Department of Biology, Faculty of Natural Sciences, University of Tirana, 1001, Tirana, Albania*

²*NanoAlb Research Center, Albanian Academy of Sciences, Tirana, Albania*

³*Center of Molecular Diagnostics and Genetic Research, University Hospital Obstetrics-Gynecologic “Mbretëresha Geraldinë”, Tirana, Albania*

⁴*Department of Medical Laboratory Technician and Imaging, Faculty of Technical Medical Sciences, Aldent University, Tirana, Albania*

*Corresponding author: E-mail: merita.xhetani@unitir.edu.al

Abstract

Breast cancer is a disease characterized by genomic alterations that influence prognosis and therapeutic response. Immunohistochemical biomarkers, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67, are routinely used for tumor classification and risk stratification. In a recent cohort of Albanian women, significant associations were observed between these biomarkers and clinicopathological parameters, with hormone receptor-positive tumors representing the predominant subtype and Ki-67 correlating with tumor stage and nodal involvement. Here, we investigate copy number variations (CNVs) and loss of heterozygosity (LOH) within the *BRCA1* and *BRCA2* genes in a selected subgroup with a family history of the disease, and integrate these findings with the evaluated prognostic biomarkers. From a retrospective cohort of 252 breast cancer patients, formalin-fixed, paraffin-embedded (FFPE) breast cancer tissue blocks from 17 patients with a documented family history were selected. DNA was extracted using the QIAamp™ DNA FFPE Tissue Kit, and genome-wide CNV profiling was performed using an Affymetrix platform. CNV and LOH, including copy-neutral LOH, were assessed and correlated with molecular subtypes defined by ER, PR, HER2, and Ki-67 expression. Copy number alterations affecting *BRCA1* and *BRCA2* were identified in 12/17 cases. *BRCA1* deletions were observed in 29.4% of tumors, while LOH at the *BRCA1* locus was detected in 47.1% of cases. *BRCA2* deletions were identified in 17.6% and LOH in 35.3% of samples. *BRCA1* alterations were predominantly associated with triple-negative breast cancer, where all cases showed evidence of genomic loss or allelic imbalance. The observed alterations in *BRCA1* and *BRCA2* highlight the importance of integrating genomic data with immunohistochemical biomarkers. These findings provide preliminary evidence of *BRCA*-associated genomic instability patterns in breast cancer patients in Albania and support the potential utility of CNV analysis to refine risk stratification and guide targeted therapeutic strategies.

Keywords: Breast cancer, Copy number variation, Loss of heterozygosity, *BRCA1*, *BRCA2*

1. Introduction

Breast cancer remains one of the most frequently diagnosed malignancies among women worldwide and is a major public health concern due to its high incidence and mortality rates. The disease is highly heterogeneous, encompassing distinct histopathological and molecular subtypes with variable clinical behavior and therapeutic responses [1]. Genomic instability is a hallmark of breast carcinogenesis. It

encompasses a broad spectrum of genetic alterations, including point mutations, chromosomal rearrangements, and copy number variations (CNVs), all of which contribute to tumor initiation and progression [1].

Among hereditary breast cancer susceptibility genes, *BRCA1* and *BRCA2* play a pivotal role because they are crucial components of the homologous recombination DNA repair pathway. Germline mutations in these genes are often linked to aggressive tumor morphologies and greatly raise the lifetime risk of breast and ovarian cancer. Prior research has shown that while *BRCA2*-related tumors more frequently resemble luminal subtypes, *BRCA1*-associated tumors are typically characterized by high histological grade, enhanced proliferative activity, and triple-negative immunophenotypes [2,3].

In routine clinical practice, immunohistochemical biomarkers, including estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2), and Ki-67, are widely used for molecular classification, prognostic assessment, and therapeutic decision-making in patients with breast cancer [4]. Integrating biomarker profiling with genomic analyses, particularly CNV assessment, may provide deeper insights into tumor biology and improve individualized patient management strategies [5].

Therefore, the present study aimed to assess copy number variations affecting *BRCA1* and *BRCA2* in a selected cohort of Albanian individuals with a family history of breast cancer and to investigate the association between genomic findings and breast cancer molecular subtypes.

2. Materials and Methods

This retrospective study included 252 breast cancer patients diagnosed and treated in Albania. From this cohort, a subgroup of 17 patients with a documented family history of breast cancer was selected for genomic analysis using their formalin-fixed, paraffin-embedded (FFPE) tumor tissue blocks.

2.1. DNA Extraction and CNV Analysis

DNA was extracted from FFPE tissues using the QIAamp™ DNA FFPE Tissue Kit according to the manufacturer's instructions. DNA was extracted using the QIAamp™ DNA FFPE Tissue Kit, and the OncoScan CNV assay was then performed, both according to the manufacturers' instructions. Briefly, genomic DNA underwent annealing to molecular inversion probes, followed by gap-fill and ligation reactions. The products were then amplified through a series of PCR steps and subjected to enzymatic digestion for quality control. Subsequently, samples were hybridized to microarray chips, followed by washing, staining, and final scanning to acquire data and enable downstream copy number analysis. Genome-wide copy number profiling was performed using an Affymetrix microarray platform. Tumors were classified according to ER, PR, HER2, and Ki-67 immunohistochemical status. Molecular subtypes were defined based on standard clinical criteria.

2.2. Statistical Analysis

Descriptive analysis was performed to evaluate the frequencies of genomic alterations and their association with molecular subtypes.

3. Results

CNVs affecting the *BRCA1* and *BRCA2* genes were identified in a considerable proportion of cases (Table 1). *BRCA1* deletions were observed in 29.4% of tumors; loss of heterozygosity (LOH) at this locus was detected in 47.1% of cases. Alterations in *BRCA2* were less frequent, with deletions identified in 17.6% and LOH in 35.3% of the samples.

Notably, *BRCA1* alterations were predominantly associated with triple-negative breast cancer, where all cases exhibited evidence of genomic loss or allelic imbalance, whereas *BRCA2* alterations were distributed across Luminal A, Luminal B, and HER2-enriched subtypes (Table 2). The majority of alterations occurred within large chromosomal segments, reflecting underlying genomic instability rather than focal gene-specific events.

Table 1. Copy number variation (CNV) and loss of heterozygosity (LOH) status of *BRCA1* (17q21.31) and *BRCA2* (13q13.1) in 17 FFPE breast cancer samples analyzed using an Affymetrix microarray platform.

No.	Sample ID	<i>BRCA1</i> CNV status	<i>BRCA1</i> LOH	Type of change <i>BRCA1</i>	<i>BRCA2</i> CNV status	<i>BRCA2</i> LOH	Type of change <i>BRCA2</i>	Comments
1	BC01	Loss	Yes	Segmental breakage	Normal	No	—	Associated with <i>TP53</i> loss <i>BRCA1</i> -related tumor
2	BC02	Normal	No	—	Normal	No	—	Stable genome (normal)
3	BC03	Normal	Yes	cnLOH	Normal	Yes	cnLOH	Genome-wide LOH pattern LOH global.
4	BC04	Loss	Yes	Focal breakage	Normal	No	—	<i>BRCA1</i> -specific event (focal)
5	BC05	Normal	No	—	Normal	No	—	Diploid
6	BC06	Normal	No	—	Loss	Yes	Focal breakage	<i>BRCA2</i> focal loss
7	BC07	Normal	Yes	cnLOH	Normal	No	—	Isolated <i>BRCA1</i> LOH
8	BC08	Loss	Yes	Segmental deletion	Normal	No	—	Large 17q deletion
9	BC09	Normal	No	—	Normal	Yes	cnLOH	Copy-neutral event
10	BC10	Normal	No	—	Normal	No	—	No detectable CNV
11	BC11	Loss	Yes	Segmental deletion	Normal	No	—	<i>TP53</i> loss <i>BRCA1</i> -related tumor
12	BC12	Normal	No	—	Loss	Yes	Focal breakage	13q loss <i>BRCA2</i> tumor related
13	BC13	Normal	No	—	Normal	No	—	Normal
14	BC14	Normal	Yes	cnLOH	Normal	Yes	cnLOH	Genome-wide LOH pattern LOH global.
15	BC15	Loss	Yes	Focal deletion	Normal	No	—	<i>BRCA1</i> -specific event
16	BC16	Normal	No	—	Normal	No	—	Diploid
17	BC17	Normal	No	—	Loss	Yes	Focal deletion	<i>BRCA2</i> focal loss

The analysis revealed diverse alterations in the *BRCA1* and *BRCA2* genes, including CNV, LOH, and cnLOH, indicative of significant molecular heterogeneity and genomic instability. CNV status is categorized as normal (diploid), loss, or gain. LOH includes both copy number-associated LOH and copy-neutral LOH (cnLOH). Segmental changes refer to large chromosomal regions, whereas focal events are confined to gene-level intervals.

Table 2. The association between the molecular classification of breast cancer and variations in the *BRCA1* and *BRCA2* genes.

ID	Molecular classification	ER	PR	HER2	<i>BRCA1</i> CNV status	<i>BRCA1</i> LOH	<i>BRCA2</i> CNV status	<i>BRCA2</i> LOH	Interpretation
BC01	Luminal A	+	+	-	Loss	Yes	Normal	No	<i>BRCA1</i> -associated instability
BC02	Luminal B	+	+	-	Normal	No	Normal	No	Genomically stable
BC03	Luminal A	+	+	-	Normal	Yes	Normal	Yes	Genome-wide LOH/cnLOH
BC04	Triple-negative	-	-	-	Loss	Yes	Normal	No	<i>BRCA1</i> specific event (focal)
BC05	Luminal B	+	+	-	Normal	No	Normal	No	Diploid, stable genome
BC06	HER2-enriched	-	-	+	Normal	No	Loss	Yes	<i>BRCA2</i> focal loss
BC07	Triple-negative	-	-	-	Normal	Yes	Normal	No	Isolated <i>BRCA1</i> LOH
BC08	HER2-enriched	-	-	+	Loss	Yes	Normal	No	Large 17q deletion
BC09	Luminal A	+	+	-	Normal	No	Normal	Yes	Copy-neutral LOH event
BC10	Luminal B	+	+	+	Normal	No	Normal	No	No detectable CNV
BC11	Triple-negative	-	-	-	Loss	Yes	Normal	No	<i>TP53</i> loss/ <i>BRCA1</i> -related
BC12	Luminal A	+	+	-	Normal	No	Loss	Yes	<i>BRCA2</i> tumor-related loss
BC13	HER2-enriched	-	-	+	Normal	No	Normal	No	Normal genome
BC14	Luminal B	+	+	-	Normal	Yes	Normal	Yes	Genome-wide LOH pattern
BC15	Triple-negative	-	-	-	Loss	Yes	Normal	No	<i>BRCA1</i> -associated
BC16	Luminal B	+	+	+	Normal	No	Normal	No	Diploid
BC17	Luminal A	+	+	-	Normal	No	Loss	Yes	<i>BRCA2</i> focal deletion

The table presents the molecular classification of breast cancer samples according to ER, PR and HER2 status, alongside *BRCA1* and *BRCA2* genomic alterations, CNV status, and LOH, with their corresponding biological and clinical interpretations.

4. Discussion

In our study, CNVs and LOH in the *BRCA1* and *BRCA2* genes were identified in a considerable proportion of the breast cancer samples analyzed. Our findings demonstrated that *BRCA1* alterations were more frequent than those observed in *BRCA2*, particularly LOH, which was detected in 47.1% of cases. This result is consistent with the existing literature, which reports high frequencies of allelic loss in the 17q21 region containing *BRCA1*, especially in tumors with high aggressiveness and defects in homologous DNA repair [6].

The predominance of *BRCA1* alterations in the triple-negative breast cancer (TNBC) subtype is consistent with extensive molecular evidence showing that TNBC is often characterized by a “BRCAness” phenotype, pronounced genomic instability, and disruption of the homologous recombination pathway. Previous studies have demonstrated that TNBC tumors more frequently exhibit deletions, LOH, and functional *BRCA1* mutations compared with hormone receptor-positive subtypes [7]. The observation in our study that all TNBC cases displayed genomic loss or allelic imbalance further supports this biological association.

BRCA2 alterations were predominantly distributed in the Luminal A, Luminal B, and HER2-enriched subtypes, suggesting that *BRCA2*-related defects may follow biological patterns distinct from those of *BRCA1*. The literature indicates that *BRCA2*-associated tumors are more often estrogen receptor-positive

and phenotypically closer to luminal breast cancers than to TNBC [8]. This is consistent with our findings, in which *BRCA2* was not predominant among triple-negative cases.

One important finding was that most alterations were localized to large chromosomal segments rather than being focal changes restricted solely to the gene level. This pattern suggests the presence of general genomic instability, which is characteristic of many aggressive breast tumors.

Genomic profiling studies using microarray and single nucleotide polymorphism (SNP) arrays have shown that segmental losses, broad amplifications, and *cnLOH* are important indicators of tumor progression and clonal heterogeneity, reflecting a high level of genomic instability in breast cancer [9].

Our research group has previously investigated recent trends in breast cancer and evaluated the clinical significance of established biomarkers, including ER, PR, HER2, and Ki-67 [10–12].

In the present study, we further expanded this line of research by examining the association between these biomarkers and *BRCA1* and *BRCA2* alterations, thereby contributing to a more comprehensive molecular characterization of breast cancer. In future studies, we aim to include a larger number of participants and incorporate broader molecular profiling approaches to further strengthen and validate these results.

5. Conclusions

In conclusion, our results suggest that alterations in *BRCA1*, particularly *LOH*, are frequent and strongly associated with the TNBC subtype, whereas *BRCA2* alterations are more commonly observed in receptor-positive tumors. The predominance of segmental changes supports the role of widespread genomic instability in the pathogenesis of breast cancer. These findings also underscore the importance of molecular analyses for prognostic classification and therapeutic decision-making.

Acknowledgements: We want to thank everyone who contributed to and supported this study.

Declaration of Generative AI and AI-Assisted Technologies: During the preparation of this manuscript, the authors used an artificial intelligence tool (ChatGPT-4o mini; OpenAI) solely for language editing, including grammar, spelling, punctuation, and clarity. The AI tool was not used to generate scientific content, analyze data, interpret results, or make substantive intellectual contributions to the manuscript. The authors reviewed and edited all AI-assisted text and take full responsibility for the accuracy, originality, and integrity of the final manuscript.

Conflict of Interest: The authors declare no conflict of interest.

Funding: This research received funding from the Albanian American Development Foundation, through the READ program (grant no. 10072026).

References

1. Weigelt B, Reis-Filho JS. Histological and molecular types of breast cancer: is there a unifying taxonomy? *Nat Rev Clin Oncol*. 2009;6(12):718–30. doi:[10.1038/nrclinonc.2009.166](https://doi.org/10.1038/nrclinonc.2009.166)
2. Rakha E, Reis-Filho JS. Basal-like breast carcinoma: from expression profiling to routine practice. *Arch Pathol Lab Med*. 2009;133(6):860–8. doi:[10.5858/133.6.860](https://doi.org/10.5858/133.6.860)
3. Rastelli F, Biancanelli S, Falzetta A, Martignetti A, Casi C, Bascioni R, et al. Triple-negative breast cancer: current state of the art. *Tumori*. 2010;96(6):875–88. doi:[10.1700/548.6505](https://doi.org/10.1700/548.6505)
4. Geyer FC, Marchiò C, Reis-Filho JS. The role of molecular analysis in breast cancer. *Pathology*. 2009;41(1):77–88. doi:[10.1080/00313020802563536](https://doi.org/10.1080/00313020802563536)
5. Thennavan A, Beca F, Xia Y, Garcia-Recio S, Allison K, Collins LC, et al. Molecular analysis of TCGA breast cancer histologic types. *Cell Genom*. 2021;1(3):100067. doi:[10.1016/j.xgen.2021.100067](https://doi.org/10.1016/j.xgen.2021.100067)

6. Watkins JA, Irshad S, Grigoriadis A, Tutt AN. Genomic scars as biomarkers of homologous recombination deficiency and drug response in breast and ovarian cancers. *Breast Cancer Res.* 2014;16(3):211. doi:[10.1186/bcr3670](https://doi.org/10.1186/bcr3670)
7. Asleh K, Riaz N, Nielsen TO. Heterogeneity of triple negative breast cancer: current advances in subtyping and treatment implications. *J Exp Clin Cancer Res.* 2022;41(1):265. doi:[10.1186/s13046-022-02476-1](https://doi.org/10.1186/s13046-022-02476-1)
8. Tung NM, Garber JE. BRCA1/2 testing: therapeutic implications for breast cancer management. *Br J Cancer.* 2018;119(2):141–52. doi:[10.1038/s41416-018-0127-5](https://doi.org/10.1038/s41416-018-0127-5)
9. Steele CD, Abbasi A, Islam SMA, Bowes AL, Khandekar A, Haase K, et al. Signatures of copy number alterations in human cancer. *Nature.* 2022;606(7916):984–91. doi:[10.1038/s41586-022-04738-6](https://doi.org/10.1038/s41586-022-04738-6)
10. Hasa A, Xhetani M, Lika M, Marku F, Dishi R, Celiku S. Correlation of prognostic biomarkers with histologic features in Albanian women with breast cancer. *J Biol Res.* 2026;99(1):13977. doi:[10.4081/jbr.2026.13977](https://doi.org/10.4081/jbr.2026.13977)
11. Xhetani M, Hasa A, Laze B. BRCA1 mutations in family members with breast cancer history [abstract EP01.030]. *Eur J Hum Genet.* 2024;32(Suppl 2):912. doi:[10.1038/s41431-024-01733-5](https://doi.org/10.1038/s41431-024-01733-5)
1. Hasa A, Xetani M, Laze B, Dishi R. BRCA1 and BRCA2 genes: key genetic drivers in breast cancer risk and management. *J Nat Sci.* 2024;36:310–23. doi:[10.70827/1224bbgkmdi](https://doi.org/10.70827/1224bbgkmdi)

How to cite: Xhetani M, Hasa A. Integrative Analysis of Genetic Variation in Breast Cancer: A Retrospective Study of the Albanian Cohort. *Eur J Med Transl Biosci.* 2026;1(1):20–25.