

NANOTECHNOLOGY-ENABLED GENE CHIP PLATFORMS FOR PRECISION BREAST CANCER GENOMICS: FIRST ALBANIAN EXPERIENCE

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ABSTRACT

Background: Recent advances in nanotechnology have transformed cancer genomics through the development of high-density DNA microarray (gene chip) platforms capable of genome-wide analysis with high analytical resolution. Technologies such as CytoScan™ 750K and OncoScan® CNV Assay integrate thousands of miniaturized oligonucleotide and molecular inversion probes on biochips, enabling comprehensive detection of copy number alterations (CNAs), copy-neutral loss of heterozygosity (LOH), and chromosomal imbalances from clinical specimens, including FFPE tissues. This study presents the first application of these nanotechnology-based genomic platforms for breast cancer characterization in Albania. **Methods:** A retrospective cohort of 252 breast cancer patients diagnosed between 2021 and 2024 was evaluated for clinicopathological characteristics. Based on hereditary risk criteria, 17 FFPE tumor samples underwent genome-wide analysis using the OncoScan® Array Chip, while the study highlights the complementary role of CytoScan™ 750K as a high-resolution genome-wide platform for constitutional and somatic genomic profiling. Genomic findings were correlated with molecular subtypes and immunohistochemical biomarkers. **Results:** The cohort was dominated by patients older than 45 years (92.5%), with invasive ductal carcinoma accounting for 81.6% of cases and stage II representing 69.8%. Genome-wide analysis revealed BRCA1 deletions in 29.4% and LOH in 47.1% of tumors, whereas BRCA2 deletions and LOH were detected in 17.6% and 35.3% of samples, respectively. BRCA1 alterations were predominantly associated with triple-negative breast cancer, while BRCA2 abnormalities were mainly observed in Luminal A, Luminal B, and HER2-enriched tumors. The high-resolution gene chip technology enabled precise identification of clinically relevant genomic alterations that are not detectable by conventional diagnostic approaches. High-density gene chip platforms based on nanotechnology represent a powerful tool for precision oncology by enabling genome-wide detection of clinically actionable genomic alterations from routine clinical samples. **Conclusions:** The complementary application demonstrates how advanced biochip technologies can support molecular diagnosis, patient stratification, targeted therapy selection, hereditary cancer risk assessment, and implementation of personalized medicine. This work establishes the first experience in Albania using nanotechnology-driven genomic microarrays for breast cancer profiling and provides a foundation for integrating advanced genomic diagnostics into routine oncological practice.

KEYWORDS: Breast cancer, CNV, BRCA1/2, Nanotechnology, Genomics

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. These genes' germline mutations are often linked to aggressive tumour morphologies and greatly raise the lifetime risk of breast and ovarian cancer. Prior research has shown that while BRCA2-related tumours more frequently resemble luminal subtypes, BRCA1-associated tumours are typically characterised 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.

MATERIAL AND METHODS

This retrospective study included 252 breast cancer patients diagnosed and treated in Albania. From this cohort, 17 patients with available FFPE tissue blocks and documented family history of breast cancer were selected for genomic analysis.

DNA Extraction and CNV Analysis

DNA was extracted from FFPE tissues using the QIAamp DNA FFPE Tissue Kit according to the manufacturer's instructions. Following DNA extraction from FFPE tissue samples, the OncoScan CNV assay workflow was performed according to the manufacturer's 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.

Statistical Analysis

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

RESULTS

Copy number variations (CNVs) affecting the BRCA1 and BRCA2 genes were identified in a considerable proportion of cases. BRCA1 deletions were observed in 29.4% of tumors, while 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, B and HER2-enriched subtypes. 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	BRCA 1 CNV status	BRCA1 LOH	Type changes BRCA1	of BRCA 2 CNV status	BRCA2 LOH	Type changes BRCA2	of Comments
1	BC01	Loss	Yes	Segmental breakage	Normal	No	—	Associated with TP53 loss BRCA1 related tumour
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	—	BRCA1 specific event (focal)
5	BC05	Normal	No	—	Normal	No	—	Diploid
6	BC06	Normal	No	—	Loss	Yes	Focal breakage	BRCA2 focal loss

7	BC07	Normal	Yes	cnLOH	Normal	No	—	Isolated BRCA1 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	—	TP53 loss BRCA1 tumor related
12	BC12	Normal	No	—	Loss	Yes	Focal breakage	13q loss BRCA2 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	—	BRCA1 specific event
16	BC16	Normal	No	—	Normal	No	—	Diploid
17	BC17	Normal	No	—	Loss	Yes	Focal deletion	BRCA2 focal loss

The analysis of the genetic samples revealed various genomic alterations in the BRCA1 and BRCA2 genes, highlighting diverse genomic alterations, including copy number losses (CNV loss), loss of heterozygosity (LOH), and copy-neutral LOH (cnLOH), indicative of significant molecular heterogeneity and genomic instability. CN 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 & BRCA2 genes.

ID	Molecular Classification	ER	PR	HER2	BRCA1 CNV Status	BRCA1 LOH	BRCA2 CNV Status	BRCA2 LOH	Interpretation
BC01	Luminal A	+	+	-	Loss	Yes	Normal	No	BRCA1- 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	BRCA1 specific event (focal)
BC05	Luminal B	+	+	-	Normal	No	Normal	No	Diploid, Stable genome
BC06	HER2- enriched	-	-	+	Normal	No	Loss	Yes	BRCA2 focal loss
BC07	Triple- negative	-	-	-	Normal	Yes	Normal	No	Isolated BRCA1 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	TP53 loss/ BRCA1-related
BC12	Luminal A	+	+	-	Normal	No	Loss	Yes	BRCA2 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	BRCA1-associated
BC16	Luminal B	+	+	+	Normal	No	Normal	No	Diploid
BC17	Luminal A	+	+	-	Normal	No	Loss	Yes	BRCA2 focal deletion

The table presents the molecular classification of breast cancer samples according to ER, PR, HER2 status, alongside BRCA1 and BRCA2 genomic alterations, including copy number variation (CNV) status and loss of heterozygosity (LOH), with their corresponding biological and clinical interpretations.

DISCUSSIONS

In our study, copy number variations (CNVs) and loss of heterozygosity (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 as 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, loss of heterozygosity (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, 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 a general genomic instability, which is characteristic of many aggressive breast tumors. Genomic profiling studies using microarray and SNP-array 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],[11],[12]. In the present study, we further expanded this line of research by examining the association between these biomarkers and BRCA1/BRCA2 alterations, thereby contributing to a more comprehensive molecular characterization of breast cancer as well as taking into consideration the ethnicity [13]. 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.

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. It underscores the importance of molecular analyses for prognostic classification and therapeutic decision-making. This application highlights the potential of advanced biochip technologies to improve molecular diagnostics, patient stratification, targeted therapeutic decision-making, hereditary cancer risk assessment, and the implementation of personalized medicine. This study represents the first experience in Albania with nanotechnology-based genomic microarrays for breast cancer profiling and provides a significant

foundation for integrating advanced genomic diagnostics into routine oncological practice and precision (personalized) cancer care.

Ethics Approval. Not applicable. This study does not involve human participants, animals, or sensitive personal data. The research is based entirely on the analysis of archived materials.

Data Sharing Statement. The datasets generated and analysed during the current study are available from the corresponding author upon reasonable request.

Authors' Contributions. **MXh and BL:** Conceptualization, Methodology, Software, Supervision, Data curation, Writing – original draft, **AH., and AT.:** Validation, Investigation, Visualization, Writing – review & editing, **AV.** and **FB.:** Software, Formal analysis, Writing – review & editing. All authors have read and agreed to the published version of the manuscript.¹ These author have contributed equally.

AI Statement. The authors declare that no AI-assisted technologies were used in the generation of the data analysis, or image processing.

Declaration of Interest. The authors declare that they have no competing financial interests.

Funding. This work was funded by AADF under the **READ program** (Research Expertise from the Academic Diaspora) grant 2025-2026 and Academy of Sciences of Albania under the research project “Të dhëna gjenetike mbi terapinë e drejtuar të kancerit të gjirit me anë të modifikimeve të Receptorit të Amygdalinës me ndihmën e Nanoteknologjisë (**GEN-NanoCRAFT**)”, approved by the Decision of the Presidency of the Academy of Sciences No. 16, dated 17 March 2025.

REFERENCES

1. Weigelt B, Reis-Filho JS. Histological and molecular types of breast cancer: is there a unifying taxonomy? *Nat Rev Clin Oncol.* 2009 Dec;6(12):718–30. doi:10.1038/nrclinonc.2009.166
2. Rakha E, Reis-Filho JS. Basal-like Breast Carcinoma: From Expression Profiling to Routine Practice. *Archives of Pathology & Laboratory Medicine.* 2009 Jun;133(6):860–8. doi:10.5858/133.6.860
3. Francesca Rastelli, Sandra Biancanelli, Amalia Falzetta, Angelo Martignetti, Camilla Casi, Romeo Bascioni, et al. Triple-negative breast cancer: current state of the art. *Tumori.* 2010 Nov 1;(2010November-December). doi:10.1700/548.6505
4. Geyer FC, Marchiò C, Reis-Filho JS. The role of molecular analysis in breast cancer. *Pathology.* 2009 Jan;41(1):77–88. doi: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 Genomics.* 2021 Dec;1(3):100067. doi: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 Jun 3;16(3):211. doi: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 Sep 1;41(1):265. doi:10.1186/s13046-022-02476-1
8. Tung NM, Garber JE. BRCA1/2 testing: therapeutic implications for breast cancer management. *Br J Cancer.* 2018 Jul;119(2):141–52. doi: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 Jun 30;606(7916):984–91. doi: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 Jan 8. doi:10.4081/jbr.2026.13977
11. Xhetani M, Hasa A, Laze B. BRCA1 mutations in family members with breast cancer history. In: Vol. 32. Berlin, Germany: European Journal of Human Genetics; 2024. p. 913–913. doi:10.1038/s41431-024-01731-7
12. Hasa A, Xetani M, Laze B, Dishi R. BRCA1 and BRCA2 genes: Key genetic drivers in breast cancer risk and management. *JNS.* 2024 Dec 3;36(2024):310–23. doi:10.70827/1224bbgkdi
13. Xhetani M, Zaimi E, Dojčáková D, Sheraj I, Mačeková S. Genetic Structure and Population Affinities of Albanians: A Regional STR-Based Analysis Within the European Context. *Genes.* 2026 Aug 31;17(9):1059. doi:10.3390/genes17091059