Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

From detection to full characterization: a germline whole-gene deletion of BRCA1 in a patient with breast cancer illustrating evolving molecular diagnostic approaches in genomics

Maria De Bonis
Agostino Gemelli University Polyclinic
Marco Fabiani
Sapienza University of Rome
Andrey Prjibelski
University of Helsinki
Paola Concolino
Agostino Gemelli University Polyclinic
Elisa De Paolis
Agostino Gemelli University Polyclinic
Giulia Maneri
Agostino Gemelli University Polyclinic
Alessia Perrucci
Agostino Gemelli University Polyclinic
Francesco Pepe
University of Naples Federico II
Domenico Vitale
University of Naples Federico II
Domenico Cozzolino
University of Naples Federico II
Jessica Evangelista
Agostino Gemelli University Polyclinic
Tatiana D'Angelo
Agostino Gemelli University Polyclinic
Gianluca Franceschini
Università Cattolica del Sacro Cuore
Maria Piane
Sapienza University of Rome
Ida Paris
Agostino Gemelli University Polyclinic
Umberto Malapelle
University of Naples Federico II
Angelo Minucci
angelo.minucci@policlinicogemelli.it

Agostino Gemelli University Polyclinic

Short Report

Keywords: BRCA1/2, Copy number variations, Germline BRCA testing, Breast Cancer, WES, WGS, MLPA, Nanopore Sequencing, Liquid biopsy, Comprehensive genomic profiling

Posted Date: November 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11491148/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background: Large genomic rearrangements (LGRs), including copy number variations (CNVs), represent important molecular targets in *BRCA1/2 (BRCA)* testing. Although next-generation sequencing combined with dedicated bioinformatics pipelines is widely used for CNV detection, challenging cases may require an integrated approach for comprehensive genomic characterization.

Methods and Results: We report a patient with breast cancer harbouring a germline deletion encompassing the entire *BRCA1* gene, which was fully characterized at nucleotide-level resolution through the integration of multiple complementary molecular approaches. Germline *BRCA* testing was used to identify the CNV, while multiplex ligation-dependent probe amplification was performed to confirm the deletion. Whole-exome sequencing demonstrated that the deletion extended beyond *BRCA1*, encompassing adjacent genomic regions. Nanopore long-read sequencing and whole-genome sequencing were subsequently used to define the deletion breakpoints, while targeted Sanger sequencing enabled their characterization at nucleotide-level resolution.

To assess the presence of loss of heterozygosity at the tissue level, comprehensive genomic profiling was performed on the patient's breast tumour. Given the unique and unusual nature of the identified CNV, we further investigated whether this genomic rearrangement could be detected in liquid biopsy using an amplicon-based *BRCA*testing approach. Available segregation data were suggestive of a *de novo* event, although this could not be conclusively demonstrated because the family study was incomplete.

Conclusions: To our knowledge, this case represents the fourth documented instance worldwide of a germline whole-gene *BRCA1* deletion and the first reported case in Italy. Our findings highlight the value of integrating long-read sequencing with complementary orthogonal approaches for the comprehensive characterization of structural variants at nucleotide resolution and support the potential utility of long-read sequencing in the molecular diagnostic assessment of *BRCA*-related CNVs.

Introduction

Germline pathogenic and/or likely pathogenic variants (P/LPVs) in *BRCA1/2 (BRCA)* genes include single-nucleotide variants (SNVs) and small insertions/deletions *(indels*). Large genomic rearrangements (LGRs) represent a clinically relevant class of deleterious genomic alterations and may involve copy number variations (CNVs), including large deletions and duplications involving one or more exons or the whole gene. All these alterations contribute to the development of hereditary breast and ovarian cancer (HBOC) syndrome [1, 2].

Next-generation sequencing (NGS) has revolutionized genetic testing by enabling the simultaneous detection of multiple types of P/LPVs in *BRCA* genes. When combined with dedicated bioinformatics pipelines that assess depth of coverage, read distribution, and uniformity, NGS can also identify CNVs indicative of LGRs [3, 5]. Multiplex ligation-dependent probe amplification (MLPA) remains the most widely used orthogonal method for confirming CNVs detected by NGS and is routinely recommended in clinical practice for their validation [6]. Additional approaches, including array comparative genomic hybridization, whole-exome sequencing (WES), whole-genome sequencing (WGS), long-read sequencing and long-range PCR, can also be used to confirm CNVs and accurately detect the genomic breakpoints [7–10].

Here, we describe a rare case of a germline LGR at the 17q21.31 locus involving the entire *BRCA1* gene. Following germline *BRCA1* testing, the rearrangement was confirmed by MLPA. WES was initially performed to estimate the genomic extent of the rearrangement and determine whether the LGR extended to neighboring genes. The WES findings prompted further investigation using WGS and Oxford Nanopore Technologies (ONT) long-read sequencing to refine breakpoint localization. Finally, Sanger sequencing was performed to characterize the rearrangement at the nucleotide level.

To investigate whether the LGR was accompanied by loss of heterozygosity (LOH) in the tumor tissue, potentially supporting its role in tumorigenesis, comprehensive genomic profiling (CGP) was performed on the patient's breast tumor.

In addition, given the distinctive nature of the LGR, its presence was investigated in a liquid biopsy (LBx) sample to assess the feasibility of detecting it in circulating tumor DNA (ctDNA).

This case highlights the pivotal role of an integrative, multi-platform molecular approach for the successful detection and characterization of complex genomic aberrations in the diagnostic setting. It also underscores the importance of specialized expertise and technical skills for the design, execution, evaluation, and interpretation of complex molecular assays.

Materials and methods

Case report

In October 2024, a 42-year-old woman presented with right breast pain. Imaging studies, including ultrasound and mammography, revealed multiple suspicious nodules in the right breast and a single lesion in the left breast. Core needle biopsy of the dominant right-sided lesion showed high-grade invasive ductal carcinoma (ER 60%, PR 30%, HER2-negative, score 1+), with an associated ductal carcinoma in situ component. Breast magnetic resonance imaging (MRI) highlighted an extensive multicentric lesion in the right breast, with suspected involvement of the skin and pectoralis muscle (6 × 4.5 × 7 cm), as well as regional lymph node involvement. The patient had no family history of BC; however, other relatives had late-onset colorectal and uterine cancers. The clinical stage was cT3N2. Following multidisciplinary evaluation, genetic counseling was recommended, with referral of the patient for germline *BRCA* testing.

The patient received neoadjuvant chemotherapy (epirubicin and cyclophosphamide followed by weekly paclitaxel), achieving a radiological complete response on MRI. In August 2025, she underwent bilateral nipple-sparing mastectomy with prophylactic contralateral surgery and implant-based reconstruction. Final pathology revealed ypT0 ypN1 disease (1/4 lymph nodes positive).

Adjuvant treatment included endocrine therapy (an aromatase inhibitor combined with a luteinizing hormone-releasing hormone (LHRH) analogue and radiotherapy. Following the identification of a pathogenic CNV in *BRCA1*, the patient underwent risk-reducing bilateral salpingo-oophorectomy, which showed no occult malignancy. After subsequent hysterectomy, the LHRH analogue was discontinued, and abemaciclib was continued in combination with endocrine therapy. The clinical and diagnostic timeline of the patient is presented in Fig. 1.

Germline BRCA testing and MLPA analysis

After receiving written informed consent for *BRCA* testing and for the use of biological samples for research purposes, germline *BRCA* status was assessed using the amplicon-based *BRCA* Devyser library preparation kit (Devyser, Stockholm, Sweden), as previously described [5].

MLPA analysis was performed as a confirmatory test using the BRCA1 SALSA MLPA Probemix P002-D1 kit (MRC Holland, Amsterdam, The Netherlands), and the result was subsequently confirmed using the BRCA1 SALSA MLPA Probemix P087-D1 kit, as previously described [8].

Variant and CNV classification was performed in accordance with the recommendations of the American College of Medical Genetics and Genomics (ACMG) [11, 12] and the *BRCA*-specific ClinGen Evidence-based Network for the Interpretation of Germline Mutant Alleles (ENIGMA) Expert Panel guidelines [13].

WES

To further delineate the genomic extent of the LGR, WES was performed to measure the deletion boundaries and assess the potential involvement of neighboring genes. Although short-read sequencing was expected to provide only an approximate estimate of the deletion, it could nevertheless help narrow down the genomic region involved and guide subsequent investigations.

WES was performed using the Illumina DNA Prep with Exome 2.5 Enrichment kit. DNA libraries were sequenced on NovaSeq 6000 platform (Illumina Inc., San Diego, CA, USA). FASTQ files were processed using the DRAGEN v4.3 pipeline (Illumina) with the GRCh38/hg38 human reference genome. Tertiary analysis was performed using the Illumina Emedgene cloud-based platform, supporting variant annotation by public databases, institutional datasets and Emedgene proprietary tools. Structural variants (SVs) and *indels* ≥ 50 bp were detected using the DRAGEN SV caller (Manta-based) from paired-end sequencing data. CNV calling was refined using an *in-house* panel of normals (PoNs), used to model baseline coverage and correct for systematic technical biases.

ONT long-read sequencing

Based on the WES findings, genomic DNA from the proband was further analyzed using ONT long-read sequencing to achieve higher-resolution characterization of the *BRCA1* rearrangement and refine breakpoint localization. Two complementary strategies were applied: targeted enrichment using real-time adaptive sampling and unbiased WGS.

For the adaptive sampling approach, 2.0 µg of genomic DNA was processed for library preparation using the ONT ligation workflow and sequenced on a MinION device equipped with an R10.4 flow cell. Basecalling was performed using ONT high-accuracy models. Adaptive sampling was configured in MinKNOW using a custom BED/coordinate target definition spanning approximately 3 Mb around *BRCA1*, including upstream and downstream flanking regions, to maximize the probability of capturing reads informative for breakpoint localization. This approach enabled real-time enrichment of molecules mapping within the selected genomic interval while rejecting off-target reads, achieving an average on-target coverage of approximately 10×.

For the WGS approach, libraries were prepared from a total of 2.5 µg of genomic DNA using the gDNA Ligation Sequencing Kit V14, according to the manufacturer's instructions. Before loading, PromethION R10.4.1 flow cells were quality-checked in MinKNOW, requiring a minimum of 5,000 active pores. A total of 68 µL of well-resuspended Library Beads was loaded into the inlet port according to the manufacturer's instructions.

Following 48 hours of sequencing, FASTQ files were generated using the proprietary ONT secondary analysis software.

Both workflows shared a common bioinformatic pipeline. Sequencing reads were aligned to the GRCh38/hg38 reference genome using minimap2, and SVs were identified using Sniffles2. The resulting aligned BAM files were subsequently visually inspected using the Integrative Genomics Viewer (IGV) to confirm SV calls and identify reads spanning the rearrangement junctions.

Breakpoint-specific PCR amplification and Sanger sequencing confirmation

To validate the putative rearrangement junction predicted from split-read alignment, breakpoint-specific PCR was performed. Primers were designed to flank the predicted junction on opposite sides, one upstream of the proximal breakpoint and one downstream of the distal breakpoint yielding a PCR product exclusively in the presence of the deletion allele, while preventing amplification from the wild-type configuration due to the large intervening distance.

Breakpoint PCR was performed using AmpliTaq Gold (Thermo Fisher Scientific, Waltham, MA, USA) with the following cycle programme: 96°C for 10 min; (96°C for 30 s, 64°C or 57°C for 30 s, 72°C for 40 s) × 35 cycles; 72°C for 8 min. Products were qualitatively assessed on 2% agarose gels with healthy

controls run in parallel, then purified using the PureLink® PCR Purification Kit (Thermo Fisher Scientific) and subjected to Sanger sequencing. Sequence traces were analysed to define the exact junction sequence and final breakpoint coordinates according to Human Genome Variation Society (HGVS) recommendations.

Tumor and liquid BRCA testing by CGP and targeted amplicon-based NGS

To assess the presence of LOH at the tissue level, CGP was performed on the patient's breast tumor using the TruSight Oncology 500 High Throughput assay (Illumina Inc., San Diego, CA, USA), as previously reported [14].

In addition, to evaluate the ability of an amplicon-based panel to detect this LGR in LBx, *BRCA* testing was performed using the Myriapod® NGS BRCA1/2 panel kit (Diatech Pharmacogenetics, Jesi, Ancona, Italy). Myriapod NGS Data Analysis Software (v5.0.11) was used for CNV analysis, employing a proprietary algorithm for CNV calling [15].

Results and discussion

Amplicon-based NGS of *BRCA* coding regions revealed no P/LPVs, whereas CNV analysis identified a heterozygous deletion encompassing the entire *BRCA1* gene (Fig. 2). MLPA confirmed the deletion, with *BRCA1*-specific probe ratios ranging from 0.40 to 0.65, supporting a heterozygous whole gene deletion. According to ACMG and ClinGen haploinsufficiency criteria, the CNV was classified as pathogenic (Fig. 2).

WES revealed a heterozygous deletion spanning 184,224-bp (GRCh38/hg38, NC_000017.11:g.43,044,350_43,228,574del), encompassing *BRCA1* and adjacent genes (Fig. 2). However, the limitations of WES, which primarily targets coding regions and relies on short-read sequencing, precluded the precise characterization of the breakpoint junctions. Nevertheless, the analysis indicated that the rearrangement extended beyond *BRCA1*, providing the rationale for further investigations.

Adaptive sampling on the MinION platform achieved an average *on-target* coverage of approximately 10× across the approximately 3 Mb target region around *BRCA1*. Despite the reduced depth typically associated with this enrichment strategy, a coverage reduction was visually evident across the CNV interval, consistent with a heterozygous deletion, and remained clearly appreciable in the long-read dataset (Fig. 3). Manual inspection of aligned reads in IGV identified a single long read (9,147 bp) with split alignment, mapping partially upstream of *BRCA1* and partially downstream of the deleted interval (8,680 bp + 467 bp), providing direct support for a deletion junction (Fig. 3). This junction-spanning read defined a narrow candidate breakpoint interval and enabled formulation of a specific hypothesis for experimental validation.

WGS on the PromethION platform yielded a mean coverage of approximately 50× across chromosome 17, enabling direct SV detection without prior enrichment. Sniffles2 independently identified a heterozygous deletion mapping to GRCh38:chr17:43,037,615_43,334,216; consistent with a heterozygous event, breakpoint-flanking regions showed an approximately 50% reduction in local coverage relative to unaffected intervals on the same chromosome. Visual inspection of the BAM file in IGV confirmed the deletion call and the presence of junction-spanning reads, fully corroborating the result obtained by adaptive sampling.

Breakpoint-specific PCR, designed based on the junction coordinates predicted from split-read alignment, confirmed a distinct rearrangement-specific amplicon of approximately 421 bp in the proband, with no corresponding product in control DNA. Sanger sequencing of the amplicon enabled exact breakpoint resolution at nucleotide level (Fig. 3). The final HGVS nomenclature was assigned according to the GRCh38/hg38 reference genome as NC_000017.11:g.43037615_43334215del; corresponding to a 296,600 bp deletion extending beyond *BRCA1* and confirming a broader genomic rearrangement within the 17q21.31 region (Fig. 3).

Finally, assessment of the rearrangement in the patient's BC using a CGP approach confirmed the presence of the *BRCA1* deletion; however, the alteration was not classified as a *deep deletion*, and consequently, no evidence of LOH was detected. Instead, CGP identified a *BRCA1* PV, *c.329dupA* p.(E111Gfs*3) (Variant allele frequency: 34.6%) (Fig. 2). The absence of a *deep deletion* call or other evidence of LOH in the CGP analysis suggests that the somatic *BRCA1* PV may have represented the second-hit event.

LBx was performed in a run including eight samples. Both the software analysis and graphical visualization identified a whole-gene deletion of *BRCA1*, further supporting the potential of this approach for detecting CNVs in *BRCA* genes (Fig. 2), as previously reported by our group in tissue samples [15].

Familial testing showed that neither the mother nor the sister carried the deletion; however, paternal testing was not available and a *de novo* origin could not be excluded.

In the present study, we report a germline deletion of *BRCA1* in an Italian patient with BC. This LGR was initially identified through *BRCA* testing, confirmed by MLPA, and comprehensively characterized using WES, WGS, long-read sequencing and Sanger sequencing.

The deletion spans 296,600 bp and encompasses the *coiled-coil domain-containing* 200 (*CCDC200*, MIM#300947*)* gene upstream of *BRCA1*, as well as the downstream region of *BRCA1*, including *Neighbor of BRCA1 Gene 2* (*NBR2*, MIM#618708), *Neighbor of BRCA1 Gene* 1 (*NBR1*, MIM#166945) and *Transmembrane Protein 106A* (*TMEM106A*) genes.

NBR2 is located in proximity to *BRCA1* on chromosome 17, and experimental evidence suggests that the two genes share a bidirectional promoter, although their transcription is independently regulated by distinct transcriptional repressors. *NBR1* was initially proposed as a candidate gene for ovarian cancer; however, no direct association with breast or ovarian cancer has been demonstrated. More recently, it has been characterized as a highly conserved

multidomain scaffold protein involved in the selective autophagic degradation of ubiquitinated proteins [16]. *TMEM106A* is implicated in several biological processes, including macrophage activation, regulation of intracellular signal transduction, and macromolecule biosynthesis. In contrast, *CCDC200*, also known as *TMEM106A-AS1*, remains poorly characterized, and its biological function is still under investigation. [17] Based on the available clinical and molecular data, and in the absence of additional relevant phenotypic features, no definitive functional contribution can currently be attributed to the additional genes encompassed by the deletion.

To date, only three families with HBOC carrying LGRs involving a complete *BRCA1* deletion have been reported (Table 1) [18, 19].

Table 1
Literature-reported whole-gene *BRCA1* CNV

Year	Molecular screening and confirmation	Segregation with disease	Mapped Breakpoints (GRCh38/hg38)	Size (bp)	Genes involved beyond *BRCA1*	Clinical Phenotype	Population/ancestry
2026	*BRCA* NGS testing/MLPA/WGS/ONT/Sanger sequencing	NA	g.43037615_43334215del	296,600	*NBR2–NBR1-TMEM106A-CCDC200*	BC	Italian
2011	*BRCA* Sanger sequencing/MLPA/array-CGH	Yes	ND	≈ 150,000	*VAT1-RND2-ΨBRCA1-NBR2*	Bilateral BC	Spanish
2008	SNP haplotype analysis/MLPA/array-CGH	Yes	ND	NA	ND	HBOC	European
2006	MLPA	Yes	ND	NA	ND	HBOC	Spanish

ND: Not Determined; NA: Not applicable; Del: deletion

Table 1. The table summarizes the four cases of whole-gene ***BRCA1*** deletion currently reported in the literature. Of note, both an evolution and an integration of genomic technologies can be observed over time. The evolution is reflected in the progressive development of the techniques used to identify and characterize these SVs: in 2006, **MLPA alone** was used to identify the CNV, followed by **Sanger sequencing** in 2011, and, more recently, **NGS,** including second- and third-generation sequencing technologies. The integration of different genomic approaches is also evident. In the 2011 case, **Sanger sequencing** was employed for *BRCA* testing, whereas in our study it was used in combination with other genomic approaches to precisely define the **breakpoints at the nucleotide level**

In 2006, De la Hoya *et al.* [18] described a Spanish family with a complete *BRCA1* deletion segregating with disease; the variant was identified by MLPA, although no additional molecular techniques were applied and breakpoints were not characterized. In 2008, Konecny *et al.* [19] reported a second case detected using SNP haplotype analysis, MLPA, and confirmatory array CGH; in this maternally inherited case, both daughters were affected. In 2011, Garcia-Casado *et al.* [7] described a *de novo* deletion encompassing *BRCA1* and flanking regions, although the rearrangement was not fully resolved at the breakpoint level. The recurrence of independent cases involving this genomic region may suggest the presence of a potential breakpoint hotspot; however, further evidence will be required to support this hypothesis.

Unlike previously reported cases, this is, to our knowledge, the first Italian case of a complete *BRCA1* whole-gene deletion characterized at nucleotide-level resolution through an integrated multi-platform genomic strategy. Although LGRs involving *BRCA1* have previously been reported in Italian patients, including the series described by Rizza *et al*.,[8] these cases did not include a complete whole-gene *BRCA1* deletion.

An additional noteworthy finding of this study was the identification of a somatic *BRCA1* PV in the absence of detectable LOH. According to Knudson's two-hit hypothesis, *BRCA* genes generally require biallelic inactivation to result in complete loss of function. In *BRCA1*-associated tumors, including BC, the second hit most commonly occurs through LOH of the remaining wild-type allele, although it can also result from the acquisition of a second PV [20]. In our patient, the identification of a somatic PV may be interpreted as a second-hit event, potentially leading to biallelic *BRCA1* inactivation in the absence of detectable LOH. This finding highlights the molecular heterogeneity of *BRCA1* inactivation and underscores the importance of integrating germline and tumor genomic profiling to accurately reconstruct the sequence of oncogenic events underlying tumor development, with potential implications for understanding tumor biology and informing therapeutic decision-making.

Finally, the detection of the whole-gene *BRCA1* deletion in ctDNA further supports the technical feasibility of using amplicon-based NGS approaches for identifying *BRCA* CNVs in plasma. However, this observation is limited to a single case and requires validation in larger studies before any conclusions regarding routine clinical application can be drawn.

The available segregation data are suggestive of a *de novo* event, although definitive confirmation was not possible because paternal testing was unavailable. This represents a limitation of the present study, as complete familial screening could not be performed to determine whether the identified structural variant was truly de novo or inherited. Although the patient's family history included late-onset colorectal and uterine cancers, no P/LPVs were identified in Lynch syndrome-associated genes by WES. Based on the available clinical and molecular evidence, these malignancies are therefore unlikely to represent *BRCA1*-related tumors and may instead represent coincidental findings.

Overall, the combined application of adaptive sampling and long-read WGS, supported by PCR/Sanger confirmation, enabled the precise characterization of this large deletion, transforming the initial CNV finding into a fully resolved SV defined at nucleotide resolution and suitable for clinical classification and reporting.

Conclusion

Most previously characterized germline LGRs involving *BRCA1* consist of intragenic CNVs, whereas whole-gene deletions are exceedingly rare, with only three cases previously reported. Here, we report the fourth case of a whole-gene germline *BRCA1* deletion and demonstrate how the integration of long-read sequencing with complementary orthogonal approaches enabled comprehensive characterization of the LGR, thereby strengthening molecular diagnostic assessment and supporting accurate clinical reporting.

These findings support the potential clinical utility of long-read sequencing for the detection and characterization of structural variants in clinically relevant genes such as *BRCA1* and *BRCA2*, particularly as these technologies move toward broader implementation in the diagnostic setting.

Abbreviations

P/LPVs
pathogenic/likely pathogenic variants
BRCA
BRCA1/2
SNVs
single-nucleotide variants
INDELS
insertions/deletions
BC
Breast cancer
LGRs
Large genomic rearrangements
CNVs
copy number variations
HBOC
hereditary breast and ovarian cancer
NGS
Next-generation sequencing
MLPA
Multiplex ligation-dependent probe amplification
WES
whole-exome sequencing
WGS
whole-genome sequencing
ONT
Oxford Nanopore Technologies
LOH
loss of heterozygosity
CGP
comprehensive genomic profiling
LBx
liquid biobsy
ctDNA
circulating tumor DNA
MRI
magnetic resonance imaging
LHRH
luteinizing hormone-releasing hormone
ACMG
American College of Medical Genetics and Genomics
ENIGMA
Evidence-based network for the interpretation of germline mutant Alleles
SVs
structural variants
PoNs

panel of normals
IGV
Integrative Genomics Viewer
CCDC200
coiled-coil domain-containing 200
NBR2
Neighbor of BRCA1 Gene 2
NBR1
Neighbor of BRCA1 Gene 1
TMEM106A
Transmembrane Protein 106A

Declarations

Competing interests.

None declared.

Ethics statements.

Written permission for the publication of the case report has been granted.

Ethics approval.

This study consisted of the description and analysis of an individual clinical case. According to the policies of Comitato Etico Lazio Area 3, ethics committee approval was not required for single case reports and therefore was not sought. All procedures performed were conducted in accordance with the ethical standards of the institution and with the Ethical Principles for Medical Research Involving Human Subjects outlined in the World Medical Association Declaration of Helsinki.

Funding.

None declared.

Author Contribution

Contributors. Conception and design: AM, MDB, GM, FP and UM; Experimental support: ADP, MF, MP; Investigations, data curation, data analysis: MP, ADP, FP, DV, DC, EDP and AP; Clinical resources: IP, TDA and GF; Writing and figure preparation: GM, AP, JE. PC and AM; Approval of final manuscript: all the authors. Guarantor: AM and UM

Acknowledgement

We thank the Genomics Core Facility Group at the Gemelli Science and Technology Park (G-STeP) for their valuable contribution.

Data Availability

This is a methodological study. All data supporting the findings of this study are available within the manuscript and additional data are available from the corresponding author upon reasonable request.

References

1. Yoshida R (2021) Hereditary breast and ovarian cancer (HBOC): review of its molecular characteristics, screening, treatment, and prognosis. Breast Cancer 28:1167–1180
2. Lynch 1 HT, Snyder C, Casey MJ (2013) Hereditary ovarian and breast cancer: what have we learned? Ann Oncol 24(Suppl 8):viii83–viii9
3. Lee JH, Ryoo N-H, Ha J-S et al (2022) Next Generation Sequencing is a Reliable Tool for Detecting BRCA1/2 Mutations, Including Large Genomic Rearrangements. Clin Lab 1;68(2)
4. Han E, Yoo J, Chae H et al (2020) Detection of BRCA1/2 large genomic rearrangements including BRCA1 promoter–region deletions using next–generation sequencing. Clin Chim Acta 505:49–54

5. Concolino P, Mello E, Minucci A et al (2014) Advanced tools for BRCA1/2 mutational screening: comparison between two methods for large genomic rearrangements (LGRs) detection. Clin Chem Lab Med 52:1119–1127
6. Minucci A, Scambia G, Santonocito C et al (2015) Clinical impact on ovarian cancer patients of massive parallel sequencing for BRCA mutation detection: the experience at Gemelli hospital and a literature review. Expert Rev Mol Diagn 15:1383–1303
7. Garcia-Casado Z, Romero I, Fernandez-Serra A et al (2011) A de novo complete BRCA1 gene deletion identified in a Spanish woman with early bilateral breast cancer. BMC Med Genet 11:12134
8. Rizza R, Hackmann K, Paris I et al (2019) Novel BRCA1 Large Genomic Rearrangements in Italian Breast/Ovarian Cancer Patients. Mol Diagn Ther 23:121–126
9. Yuan N, Jia P (2024) Comprehensive assessment of long-read sequencing platforms and calling algorithms for detection of copy number variation. Brief Bioinform 25(5)
10. Tilemis FN, Marinakis NM, Veltra D et al (2023) Germline CNV Detection through Whole-Exome Sequencing (WES) Data Analysis Enhances Resolution of Rare Genetic Diseases. Genes 14:1490
11. Richards S, Aziz N, Bale S et al (2015) Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med 17:405–424
12. Riggs ER, Andersen EF, Cherry AM et al (2020) Technical standards for the interpretation and reporting of constitutional copy-number variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Clinical Genome Resource (ClinGen). Genet Med 22:245–257
13. Parsons MT, de la Hoya M, Richardson ME et al (2024) Evidence-based recommendations for gene-specific ACMG/AMP variant classification from the ClinGen ENIGMA BRCA1 and BRCA2 Variant Curation Expert Panel. Am J Hum Genet 111:2044–2058
14. Nero C, Duranti S, Giacomini F et al (2022) Integrating a Comprehensive Cancer Genome Profiling into Clinical Practice: A Blueprint in an Italian Referral Center. J Pers Med 12:1746
15. Perrucci A, De Bonis M, Maneri G et al (2025) Detection of Clinically Significant BRCA Large Genomic Rearrangements in FFPE Ovarian Cancer Samples: A Comparative NGS Study. Genes 16:1052
16. Campbell IG, Nicolai HM, Foulkes WD et al (1994) A novel gene encoding a B-box protein within the BRCA1 region at 17q21.1. Hum Mol Genet 3:589–594
17. Lange S, Xiang F, Yakovenko A et al (2005) The kinase domain of titin controls muscle gene expression and protein turnover. Science 308:1599–1503
18. de la Hoya M, Gutiérrez-Enríquez S, Velasco E et al (2006) Genomic rearrangements at the BRCA1 locus in Spanish families with breast/ovarian cancer. Clin Chem 52:1480–1485
19. Konecny M, Zavodna K, Vranova V et al (2008) Identification of rare complete BRCA1 gene deletion using a combination of SNP haplotype analysis, MLPA and array-CGH techniques. Breast Cancer Res Treat 109:581–583
20. Wang LH, Wu CF, Rajasekaran N et al (2018) Loss of Tumor Suppressor Gene Function in Human Cancer: An Overview. Cell Physiol Biochem 51:2647–2649

Figures

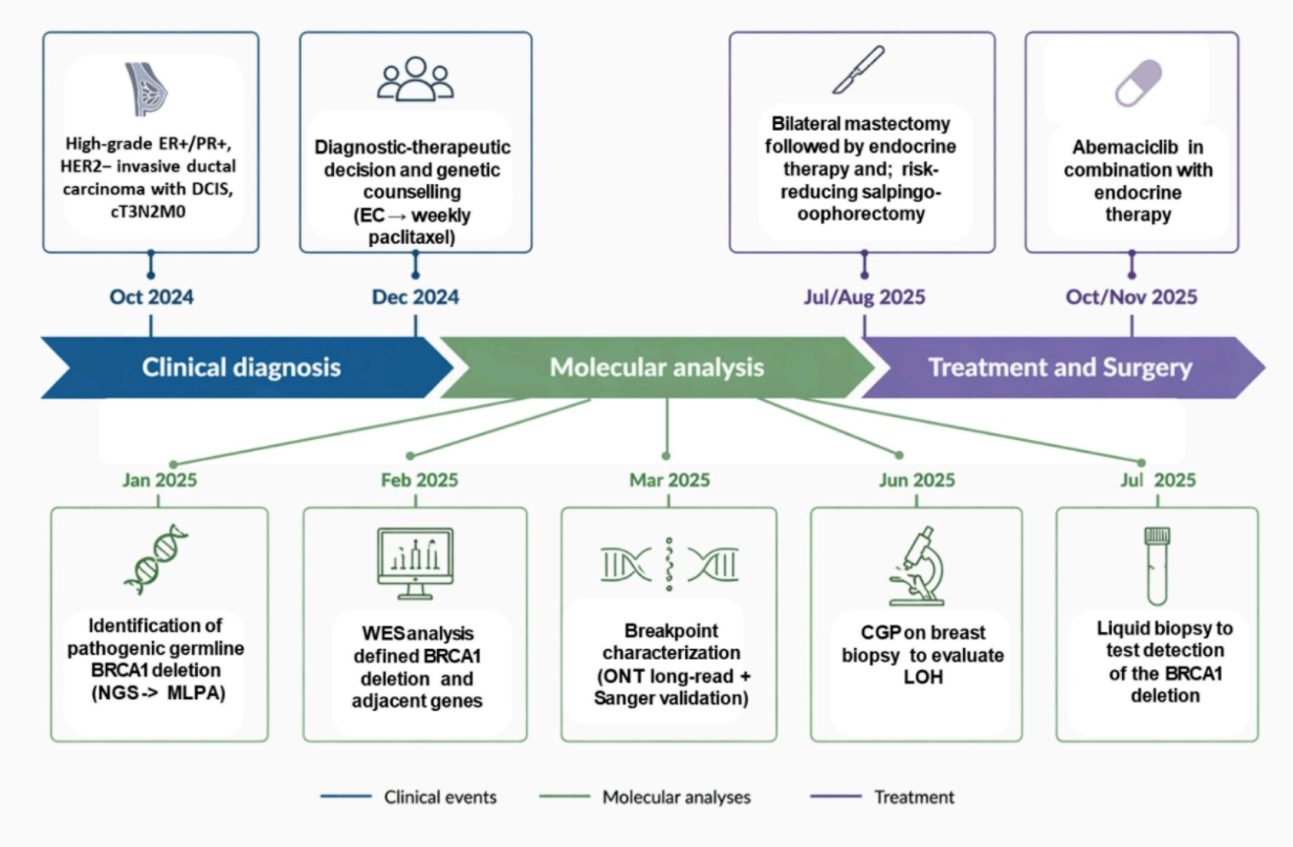


Figure 1

Clinical timeline and integrated molecular analyses. Overview of the patient's clinical course and treatment, integrated with sequential molecular analyses used in this study to identify and characterize the structural alteration at the genomic level



Figure 2

Multimodal molecular characterization of a whole-gene *BRCA1*deletion. The figure shows the analytical strategies adopted for the characterization of the *BRCA1* rearrangement. **Panel A.** NGS germline-based CNV analysis. Data analysis detected the *BRCA1*whole-gene deletion. The plot displays normalized read-depth ratios for each targeted exon across the *BRCA* genes. A heterozygous large deletion encompassing the entire *BRCA1* gene is indicated by red data points, showing reduced normalized copy number values (approximately 0.5), consistent with single-copy loss. BRCA2 exons show normalized values around 1, indicating normal diploid copy number and are represented by gray points (high confidence). FASTQ files were analyzed with the CE-IVD Amplicon Suite Software v3.87.0 (SmartSeq, Novara, Italy). **Panel B**. MLPA results. (left) Fragment analysis profile. All peaks corresponding to *BRCA1* exons display an intensity approximately half that of the reference probe peaks. (right) MLPA results showing final ratio values for all *BRCA1*probes ranging between 0.40 and 0.65, consistent with a heterozygous whole-gene deletion. The following cut-off values for the final probe ratio (FR) were applied for MLPA result interpretation: 0 copies (FR = 0), 1 copy (0.40 < FR < 0.65), 2 copies (0.80 < FR < 1.20), and 3 copies (1.30 < FR < 1.65). **Panel C.** WES results. Genome view from Emedgene analysis software showing CNV overlapping four protein-coding genes. **Panel D**. IGV view and variant coordinates from Velsera analysis software. Data analysis from somatic sequencing identified a *BRCA1*loss (left) and an oncogenic *BRCA1*variant (right). **Panel E**. Copy Number evaluation from liquid biopsy analysis. The figure shows the CNV prediction of *BRCA1* (left) and *BRCA2* (right). The distribution of normalized amplicon coverages of *BRCA1*clearly shows the whole gene loss of BRCA1 gene (score 0.5) when compared with the data from *BRCA2* (score 1.0).



Figure 3

***BRCA1* CNV characterization by WES, long-read and Sanger sequencing.** Comprehensive molecular characterization of an entire *BRCA1* gene deletion. **Panel A**. Genome browser of the *BRCA1* locus showing the concordance between short-read CNV detection and long-read sequencing coverage. The light-blue bar indicates the CNV region inferred from WES CNV calling. The track below reports the ONT adaptive sampling depth-of-coverage, highlighting a clear coverage depletion within the same interval compared with adjacent regions, consistent with a heterozygous large deletion affecting the *BRCA1* genomic region. **Panel B**. Breakpoint Mapping and Validation. Detailed characterization of the exact deletion breakpoints. **Panel B1** shows the in-silico breakpoint identification: zoomed-in IGV views revealing split and reads (highlighted in red boxes) that span the deletion breakpoints. The schematic diagram below illustrates the structural rearrangement at the junction, showing the fusion of the two flanking genomic segments, risultante da una singola read di 9.147 bp. The read aligns upstream of BRCA1 for 8,680 bp, and the remaining 467 bp aligns downstream of the deleted interval, consistent with a deletion junction bringing the two distant genomic regions. The highlighted sequence block indicates the predicted breakpoint-containing segment used to guide junction-specific primer design for orthogonal validation. The bottom panels display the nucleotide-level read alignments precisely pinpointing the genomic breakage points. **Panel B2** shows the PCR validation: design and execution of a junction-specific PCR. The left panel shows the in-silico reconstructed sequence of the mutant allele at the junction, highlighting the annealing sites for the forward (Primer F, green) and reverse (Primer R, cyan) primers, which are designed to yield an expected amplicon of 421 bp. The right panel displays the agarose gel electrophoresis of the PCR products, confirming the presence of the specific 421 bp band exclusively in the patient's sample (*BRCA1*-Deletion) and its complete absence in the wild-type control at both 64°C and 57°C annealing temperatures. **Panel B3**shows the Sanger sequencing electropherogram of the 421 bp junction-specific PCR product, definitively confirming the exact breakpoint junction, enabling final HGVS nomenclature was assigned according to the GRCh38/hg38 reference genome as NC_000017.11:g.43037615_43334215del; corresponding to a 296,600 bp deletion extending beyond *BRCA1*.