Recurrent, Cryptic *BRCA1* Complex Rearrangement by Whole-genome Sequencing in Pancreatic and Breast Cancer Patients

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BACKGROUND

Germline pathogenic variants in *BRCA1* are a major cause of hereditary breast, ovarian, and pancreatic cancer syndromes. Detecting these variants is essential not only for accurate risk assessment and preventive strategies within affected families but also for clinical management, as *BRCA1* status serves as a predictive biomarker for response to poly(ADP-ribose) polymerase (PARP) inhibitor therapy.

Current standard-of-care genetic testing primarily relies on targeted panel sequencing. While effective for detecting single-nucleotide variants (SNVs) and small insertions or deletions (indels) within predefined genomic regions, these approaches have limited ability to capture large-scale genomic rearrangements, complex inversions, and other structural variations (SVs). Such events frequently occur in intronic or intergenic regions that are poorly covered by targeted assays, creating a diagnostic gap in which patients harboring pathogenic SVs may receive false-negative results. Whole-genome sequencing (WGS) addresses this gap by providing uniform, genome-wide coverage that enables sensitive detection of the full spectrum of genetic variation, including complex genomic rearrangements (CGRs) missed by targeted approaches.

Here, we present two clinical cases in which WGS identified an identical **germline *BRCA1* CGR** that was **undetected by conventional panel testing**, underscoring the immediate clinical value of WGS and its broader implications for precision oncology.

METHODS

Patients:
Fully informed consent was obtained from all patients. The study was approved by the IRB of SNUBH and SMC.

Genetic Tests used in this study:

Hereditary Cancer Panel (Targeted Panel Sequencing):
- Sequencing platform: Ion Torrent S5 XL

CancerVision™ (Whole Genome Sequencing):
- Samples: Matched fresh-frozen tumor and normal blood.
- Sequencing platform: Illumina NovaSeq6000 (40x tumor, 20x normal coverage)

MLPA for confirmation:
- SALSA MLPA Probemix P002 *BRCA1* (MRC Holland, Amsterdam, The Netherlands)
- GeneMarker software (SoftGenetics, State College, PA, USA)

CASES REPRESENTATION

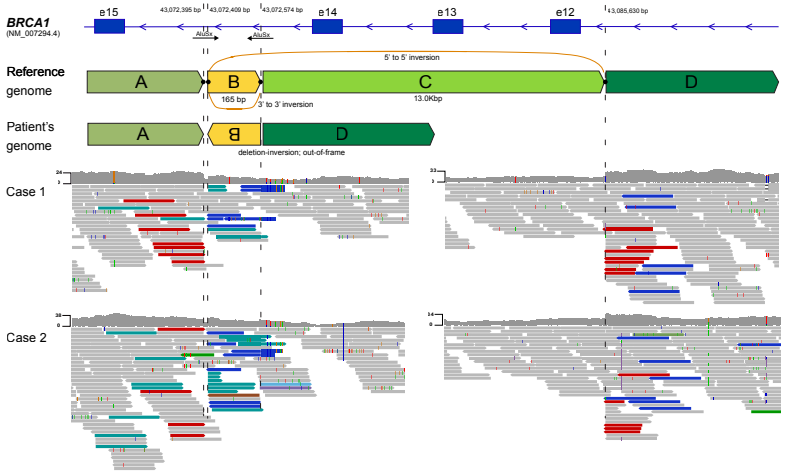


Figure 1. Large deletion and inversion in the *BRCA1* gene. Compared to the reference genome, these patients exhibited a 13.0 Kbp deletion and an adjacent inversion (165 bp). The breakpoints were precisely identical in both cases.

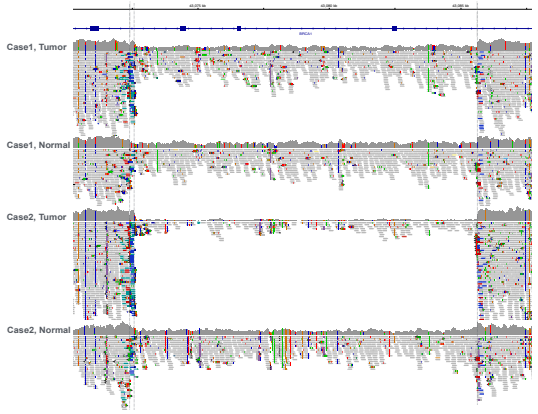


Figure 2. Wide-view IGV snapshots of *BRCA1*. Identical CGRs (large deletion of exons 12-14 and adjacent inversion) are seen in the germline (Normal WGS) of both Case 1 and Case 2. The deletion appears enhanced in Tumor WGS due to **Loss of Heterozygosity (LOH)**, confirming that the cancer cells lost the wild-type *BRCA1* allele. Breakpoints are precisely identical across these unrelated cases.

Case 1: Identification of a cryptic *BRCA1* complex genomic rearrangement (CGR) in a pancreatic cancer patient
A 61-year-old female with metastatic pancreatic ductal adenocarcinoma (PDAC) and a history of breast cancer (age 43) showed exceptional sensitivity to FOLFIRINOX, achieving complete radiographic remission and remaining stable for 38 cycles. Due to the two primary malignancies and platinum sensitivity, initial targeted germline testing was performed, but no pathogenic alterations were identified. To fully characterize the case, comprehensive WGS (CancerVision™) on matched normal tissue was applied, which revealed a cryptic germline *BRCA1* CGR. This CGR, a large deletion and inversion that deleted exons 12–14, is a pathogenic allele predicting premature protein truncation. The deletion was orthogonally confirmed by MLPA.

Case 2: Recurrence of the *BRCA1* CGR in an unrelated breast cancer patient
The second case involved a 40-year-old female diagnosed with breast cancer who was concurrently found to have Stage IB ovarian serous carcinoma. Despite the young age of onset and multiple primary cancers, initial genetic testing using BRCA1/2-targeted Sanger sequencing yielded wild-type results. Retrospective WGS (CancerVision™) analysis of archived germline tissue successfully identified the *BRCA1* CGR, confirming it was identical to the germline alteration detected in Case 1.

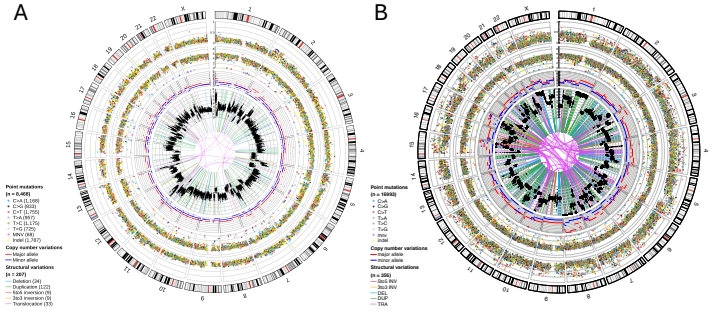


Figure 3. Circos plots of the cancer genomes for Case 1 (A) and Case 2 (B) revealing the signature of Homologous Recombination Deficiency. The plots prominently show **genome-wide allelic imbalance** (allelic copy numbers in red/blue) and a high frequency of **tandem duplications** (colored lines), which are specific genomic hallmarks of *BRCA1*-deficient cancers. This confirms the functional inactivation of *BRCA1* caused by the CGR.

DISCUSSION

This case series demonstrates that WGS is essential for resolving clinically significant genetic cases that standard targeted methods miss. The central finding was the identification of an identical, previously unknown **Complex Genomic Rearrangement** in the *BRCA1* gene in two unrelated patients.

•**Failure of Targeted Panels:** Standard **targeted panel sequencing** (useful for SNVs and small indels) often fails to detect complex, intergenic, or intronic variants like CGRs. This technical limitation can cause **false-negative results**, delaying access to crucial targeted therapies (e.g., PARP inhibitors) and impeding accurate risk assessment.

•**WGS Advantage:** WGS overcomes these limitations by providing **uniform, genome-wide coverage**, enabling the simultaneous and unbiased detection of a diverse array of genomic variations, including both simple and complex structural variants (SVs).

•**Biological Mechanism:** The deletion of *BRCA1* exons 12-14 removes a domain that interacts with *ATM*, substantially disrupting the protein complex responsible for homologous recombination. Previous deletions involving these exons were reported (see the table below), but they were not identical to this CGR, suggesting that *BRCA1* structural variants are varied and potentially under-detected by conventional testing. Of note, repetitive sequences, such as Alu elements, are enriched in *BRCA1* introns, which may lead to relatively frequent genomic rearrangements through **non-allelic homologous recombination** mechanisms. Breakpoint analysis from WGS confirmed that the CGR breakpoints overlapped with ***Alu*** elements, supporting this notion.

The findings suggest that structural variants in *BRCA1* may be more common and varied than previously understood, reinforcing the need for WGS to ensure a comprehensive molecular diagnosis and optimal clinical management.

Author	Variant type (size)	Involved Alu at intron 14	Involved Alu at intron 11
Lim et al, 2007[1]	Deletion (10,411bp)	AluSx (-)	AluSx (-)
Seong et al, 2009[2]	Deletion (11,467bp)	AluSp (-)	AluY (-)
This study	Deletion (13,056 bp) with inversion (165 bp)	AluSp (+)* and AluSp (-)**	None

*5' end of inversion; **5' end of deletion

1. Lim et al. (2007). Identification of novel *BRCA* large genomic rearrangements... *Clin Genet.*
2. Seong et al. (2009). Comprehensive mutational analysis of *BRCA1/BRCA2* for Korean breast cancer patients... *Clin Genet.*