New Germinal Mutation of BRCA1 gene (c.829_832delAATA) in a Polish Patient with Breast/Ovarian Cancer

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Research Article

Keywords: BRCA1, BRCA2, ovarian/breast cancer, NGS, PARP inhibitors

DOI: https://doi.org/10.21203/rs.3.rs-126547/v1

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Abstract

**Background:** Determination of the BRCA1/BRCA2 mutation status in patients with breast and/or ovarian cancer is commonly performed using various molecular techniques. The use only of targeted PCR-based tests may not be sufficient, as not all possible variants are investigated. Quick and effective diagnosis in order to apply the appropriate treatment is extremely important. In the following study, we used next generation sequencing (NGS) techniques to identify novel pathogenic variants in BRCA1 and BRCA2.

**Methods:** In this study, material (blood and FFPE) collected from a 67-year-old patient with ovarian cancer was used. The presence of hereditary mutations characteristic for the Polish population was examined using Sanger sequencing. BRCA1 and BRCA2 gene exons were amplified using the Devyser BRCA kit and sequenced on the Miniseq.

**Results:** No germline mutations characteristic for the Polish population were detected. However, 12 single nucleotide variants and 2 indels were identified. We found a new deleterious mutation of gene BRCA1 (c.829_832delAATA). To our knowledge, this mutation has not been reported yet in the Polish population and others.

**Conclusions:** The use of the NGS technique increases the possibilities of detecting mutational changes in patients with ovarian and/or breast cancer. The frequency of somatic mutations in ovarian tumors is low (3% - 9%) but their detection may have therapeutic benefits due to the use of poly(adenosine diphosphate)-ribose polymerase (PARP) inhibitors. Quick determination of pathogenic variants is important to facilitate specific therapy in addition to the identification of familial predisposition to cancer.

**Background**

Ovarian cancer was the eighth frequently occurring in women and 18th of all in the world in 2018 [1]. In 2018, there were nearly 295 400 new cases of ovarian cancer in the World and 5077 in Poland [2]. Approximately, 15% of ovarian cancers are caused by germline BRCA1/2 (Breast cancer type 1 susceptibility protein and Breast cancer type 2 susceptibility protein) genes mutations [3], while 3–9% are due to somatic pathogenic variants [4].

BRCA1 gene (OMIM#13705) is located on chromosome 17q12, and it encodes a multifunctional protein involved in DNA homologous recombination repair [5], G2 cell cycle checkpoint regulation [6], cell survival, and chromosome stability [7]. RING finger domain binds Bard1.p and Ola1.p forming E3 ubiquitin ligase complex [8].

Germline mutations of BRCA1 gene have been detected in the majority of familial breast and ovarian cancers. Additionally, 30–40% of sporadic cases are associated with altered expression of BRCA1 [9]. In the Polish population, the most commonly studied mutations in the BRCA1 gene are: 5382insC (c.5266dupC; p.Gln1756Profs), 300T > G (c.181T > G; p.Cys61Gly), 185delAG (c.68_69delAG; p.Glu23Valfs), 4153delA (c. 4035delA; p.Glu1346Lysfs) [10, 11].
BRCA1 mutations associated with breast cancers are usually basal-like and triple negative without HER2 gene amplification [12]. The risk of breast cancer for BRCA1 and BRCA2 germline mutations among the carriers under 80 years is about 72% and 69% respectively. Similarly, the risk of serous ovarian cancer is 44% (for BRCA1) and 17% (for BRCA2) [13, 14]. Homozygous nonfunctional mutations of BRCA1 usually result in embryo lethality. Biallelic compound heterozygous variant of BRCA1 containing c.594_597del4 and c5095C > T is viable but exhibits susceptibility to microsomia, dysmorphia, mild intellectual disability, crosslinking agents, and breast cancer [15].

Here, we present a case report on serous ovarian cancer in a 67-old female patient, including a novel germline mutation, not yet described neither in Polish nor in any other population.

Materials And Methods

The examined material included blood cells (BC) and formalin-fixed paraffin embedded tissue (FFPE) collected during standard laboratory procedures. The histological diagnosis of ovarian serous carcinoma and the tumor tissue was evaluated by pathologist.

The patient, a 67-year-old female underwent right-sided mastectomy for breast cancer (NST, G2, T2N0M0) in 2011 and complementary chemotherapy (FEC) in 2012. Furthermore, she suffered hypogastric pain in July 2017 while ascites and tumors of the left and right adnexa were detected in September of the same year. The level of CA125 was 14749 U/ml. The use of standard tests did not reveal the presence of foundational mutations in BRCA1/BRCA2 characteristic of the Polish population.

The patient was treated with chemotherapy: 6 courses of Carboplatin AUC5 and Paclitaxel which brought significant regression of the symptoms. In April 2018, the patient underwent radical surgery i.e. bilateral ovariectomy and hysterectomy. Diagnosis: G2 ovarian serous cancer with metastases to the abdomen. In November 2018, the disease progression was recorded. In January 2019, she was reoperated on and second-line chemotherapy implemented: 6 courses of Carboplatin and Paclitaxel with complete response in imaging and marker tests. BRCA1/2 mutation tests in archival material were ordered. A new pathogenic BRCA1 gene mutation was found using NGS technology. The patient was qualified for maintenance therapy with Olaparib (800 mg daily).

Approval for this study was obtained from the Bioethical Commission in Lublin (approval number 10/2020/KB/VIII). All procedures were performed in accordance with the Declaration of Helsinki (ethical principles for medical research involving human subjects).

DNA extraction and genotyping

Genomic DNA was extracted from the whole blood using commercial kit NucleoSpin Dx Blood (Machery-Nagel, Duren, Germany) according to manufacturer’s instructions. Quantity and quality of DNA was determined with the use of BioPhotometer (Eppendorf, Hamburg, Germany). Three mutations of BRCA1
gene: 5382insC (c.5266dupC), 300T>G (c.181T>G), 4153delA (c.4035delA) were amplified by PCR using the primers and under conditions described by [16,17]. For other mutations: 185delAG (c.68_69delAG; p.Glu23Valfs), 3819delGTAAA (c.3700_3704delGTAAA; (p. Val1234Glnfs), 3875delGTCT (c.3756_3759delGTCT; p.Ser1253Argfs), 3896delT (c.3779delT; p.Leu1260Tyrfs), 4160delAG (c.4041_4042delAG, p.Gly1348Asnfs) and 4184delTCAA (c.4065_4068delTCAA; p.Asn1355Lysfs) primers were designed and reaction conditions were experimentally determined (Table S1).

Primer design was performed using freely available Software Primer [18]. The Taq PCR Master Mix kit (EURx, Gdansk, Poland) was used to prepare the PCR reaction (with 40 – 60 ng DNA) in SimplyAmp™ thermal cycler (Applied Biosystems, ThermoFisher Scientific). PCR products were enzymatically purified using ExoBap kit (EURx, Gdansk, Poland). The sequential PCR was then prepared using BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). In the next step, terminators were removed after PCR using ExTerminator (A&A Biotechnology, Gdynia, Poland). After the purification. all samples were placed onto a 96-well plate and sequenced using 3100 Capillary Sequencer (Applied Biosystems, ThermoFisher Scientific). Then, the data files of the samples were checked by FinchTV1.4 (Geospiza) and compared with reference sequences BRCA1: NM_007294.3.

**MLPA assay**

BRCA1/2 genomic arrangements were searched by multiplex ligation-dependent probe amplification (MLPA) method [19] according to the manufacturer's instructions (MRC-Holland, Amsterdam, the Netherlands) using P087 SALSA and P090 SALSA MPLA kit. The products of MPLA reactions were 10 times diluted in Hi-Di™ Formamide (Applied Biosystems) and GeneScan™ 500 ROX™ dye Size Standard (Applied Biosystems) was added to each sample (as an internal lane size standard). It enabled automated data analysis. Moreover, it was essential for achieving high run to ensure precision in sizing DNA fragments by electrophoresis. The samples were size-separated by capillary electrophoresis (POP7 polymer, ABI PRISM 3100, Applied Biosystems). Thus, obtained electrophoretograms were analyzed by GeneMarker software (version 2.2.0, SoftGenetics, State College, PA, USA).

**Next Generation Sequencing**

The amount of cancer cells (evaluated by pathologist) was approximately 100% in the sample of FFPE. DNA was extracted using GeneRead DNA FFPE (QIAGEN, Hilden, Germany). The DNA concentration was determined using Qubit I fluorometer and dsDNA High Sensitivity Assay Kit (Invitrogen™, ThermoFisher Scientific).

The DNA was diluted to a concentration of 10 ng/µl. The libraries were prepared using Devyser BRCA kit (Hagersten, Sweden) according to the manufacturer's instructions. Complete sequence determination of all coding exons and all adjacent exon-intron boundaries (minimum 20 bp proximal to 5' end and 10 bp
distal to 3’ end of each exon) were covered. The first, amplicon libraries were generated in one multiplex PCR reaction (PCR1). Next, index addition to PCR1 was performed in PCR2. Sample libraries obtained in PCR2 were pooled and purified in a single tube. The library concentration was quantified using Qubit fluorometer and dsDNA High-Sensitivity Assay Kit (Invitrogen, Thermo Fisher Scientific). The purified library pool was diluted to 0.4 ng/ml concentration and denatured.

Sequencing was performed on MiniSeq (Illumina, San Diego, USA) using Mid Output Reagent Cartridge 300 cycles (Illumina, San Diego, USA). The Devyser BRCA libraries were sequenced in a paired-end mode (2 x 151 bp). The results of sequencing data files (FASTQ) were reviewed using Amplicon Suite (Smartseq s.r.l).

**Data analysis**

All mutations were reported following the Human Genome Variation Society guidelines (http://varnomen.hgvs.org/) on the basis of the coding sequences NM_007294.3 for BRCA1 and NM_000059.3 for BRCA2. For the detection of sequence variants of somatic origin, we ensured that each amplicon had at least 1,000 coverage for detection of VAF down to 5%. Mutations were classified according to clinical variants (https://www.ncbi.nlm.nih.gov/clinvar/), mutations in COSMIC database (https://cancer.sanger.ac.uk/cancergenome/projects/cosmic/), variants of unknown significance, literature search and *in silico* analyses using Varsome (https://varsome.com/). The presence of BRCA1/2 genes mutation was confirmed by Sanger sequencing (Table S1).

**Results**

Nine mutations in BRCA1 were genotyped, i.e. c.5266dupC (p.Gln1756Profs), c.181T > G (p.Cys61Gly), c.68_69delAG (p.Glu23Valfs), c. 4035delA (p.Glu1346Lysfs), c.3700_3704delGTAAA (p. Val1234Glufs), c.3756_3759delGTCT (p.Ser1253Argfs), c.3779delT (p.Leu1260Tyrfs), c.4041_4042delAG (p.Gly1348Asnfs) as well as c.4065_4068delTCAA (p.Asn1355Lysfs) and not detected in this sample.

MLPA analysis was used to test the presence of large genetics rearrangements (LGRs) in the BRCA1 and BRCA2. No deletions or duplications of genomic DNA fragments - copy number variations (CNV) were detected.

The data coverage for FFPE sample showed a mean amplicon reading depth per sample ramping from 9813 to 2690 for BRCA1 and from 17980 to 3237 for BRCA2. Seven single nucleotide variants (SNVs) and one deletion were identified in BRCA1 gene by NGS, whereas five SNVs and one deletion in BRCA2 were detected (Table 1).
Table 1

BRCA1 and BRCA2 variants detected by NGS

<table>
<thead>
<tr>
<th>c.DNA</th>
<th>Protein</th>
<th>Type</th>
<th>RS number</th>
<th>Classification</th>
<th>% reads</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BRCA1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.2612C&gt;T</td>
<td>p.Pro871Leu</td>
<td>SNV</td>
<td>rs799917</td>
<td>benign [class 1]</td>
<td>5.96</td>
</tr>
<tr>
<td>c.4308T&gt;C</td>
<td>p.Ser1436=</td>
<td>SNV</td>
<td>rs1060915</td>
<td>benign [class 1]</td>
<td>5.58</td>
</tr>
<tr>
<td>c.3113A&gt;G</td>
<td>p.Glu1038Gly</td>
<td>SNV</td>
<td>rs16941</td>
<td>benign [class 1]</td>
<td>5.85</td>
</tr>
<tr>
<td>c.2311T&gt;C</td>
<td>p.Leu771=</td>
<td>SNV</td>
<td>rs16940</td>
<td>benign [class 1]</td>
<td>5.94</td>
</tr>
<tr>
<td>c.2082C&gt;T</td>
<td>p.Ser694=</td>
<td>SNV</td>
<td>rs1799949</td>
<td>benign [class 1]</td>
<td>6.23</td>
</tr>
<tr>
<td>c.3548A&gt;G</td>
<td>p.Lys1183Arg</td>
<td>SNV</td>
<td>rs16942</td>
<td>benign [class 1]</td>
<td>6.63</td>
</tr>
<tr>
<td>c.4837A&gt;G</td>
<td>p.Ser1613Gly</td>
<td>SNV</td>
<td>rs1799966</td>
<td>benign [class 1]</td>
<td>5.69</td>
</tr>
<tr>
<td>c.829_832delAATA</td>
<td>p.Asn277Leufs20</td>
<td>del</td>
<td></td>
<td>Pathogenic [class 5]</td>
<td>94.75</td>
</tr>
<tr>
<td><strong>BRCA2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>c.68-7delT</td>
<td>del</td>
<td></td>
<td>rs276174878</td>
<td>Uncertain significance [class 3]</td>
<td>16.58</td>
</tr>
<tr>
<td>c.6513G&gt;C</td>
<td>p.Val2171=</td>
<td>SNV</td>
<td>rs206076</td>
<td>benign [class 1]</td>
<td>99.29</td>
</tr>
<tr>
<td>c.7806-14T&gt;C</td>
<td>SNV</td>
<td>rs9534262</td>
<td>benign [class 1]</td>
<td>50.87</td>
<td></td>
</tr>
<tr>
<td>c.7397T&gt;C</td>
<td>p.Val2466Ala</td>
<td>SNV</td>
<td>rs169547</td>
<td>benign [class 1]</td>
<td>99.4</td>
</tr>
<tr>
<td>c.3807T&gt;C</td>
<td>p.Val1269=</td>
<td>SNV</td>
<td>rs543304</td>
<td>benign [class 1]</td>
<td>50.93</td>
</tr>
</tbody>
</table>

All detected SNVs of BRCA1 were reported in ClinVar as benign (class 1). In this case one pathogenic variant in BRCA1 was identified: c.829-832delAATA, pAsn277Leufs*20, NM_007294. This genetic variant is frame-shift and resulted in a premature stop codon and protein truncation. This mutation is located in exon 10 of 23 (159–162 of 3426 coding, NMD).

To our knowledge, this mutation has not been reported yet in the Polish population and others. Additionally, this variant was fully confirmed by Sanger sequencing, therefore estimated specificity was 100% (Fig. 1).

Five detected BRCA2 SNVs were reported as benign (class 1). One variant c.68-7delT was reported as conflicting interpretation of pathogenicity (class 3) (Table 1).

Discussion
Determining the status of mutations in *BRCA1/2* genes is important to identify a predisposition to familial cancer as well as to select proper therapy. Hereditary mutations in the *BRCA1* and *BRCA2* genes are risk factors for ovarian cancer. Especially drugs based on PARP inhibitors and platinum can be used in patients with such mutations. New techniques can be used to analyse not only germinal but also somatic gene variants.

In *BRCA1* one deletion c.829_832delAATA was detected by NGS. According to the ACMG guidelines [20, 21], this mutation may be classified as pathogenic, fulfilling PVS1 criteria (pathogenic, very strong), PM2 (pathogenic moderate) or PP3 (supporting) and associated with breast and ovarian cancer. Pathogenic PVS1 criterion means null variant (frame shift) which is a known mechanism of disease. *BRCA1* gene has about 2,908 known pathogenic variants (which is greater than minimum of 0.7) associated with breast-ovarian cancer, familial, susceptibility to, 1, pancreatic cancer, susceptibility to, 4 and Fanconi anemia, complementation group S [20, 21].

PM2 criterion means absent in controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project or Exome Aggregation Consortium. Variants not found in GenomAD exomes coverage = 62.4) and not found in GnomAD genomes (good GnomAD genomes coverage = 32.2). PP3 pathogenic verdict computational was defined on 1 prognosis (GERP) vs no benign. Germline *BRCA1* mutation c.829_832delAATA was confirmed as well (Fig. 1).

*BRCA2* analysis in the FFPE sample by NGS showed five SNV-type variations and one deletion. The c.68_7delT variant was not precisely qualified according to the ClinVar database. Two of the four annotations describe this variant as of uncertain significance (VUS) (class 3) according to ACMG Guidelines [20], and the other two as benign (class 1) variants [21]. The https://varsome.com/database based on the American College of Medical Genetics and Genomics Classification indicates that variant as a change of unknown significance (class 3) [21].

In this case, no mutation previously described as Polish-founder mutation was identified. NGS based testing allowed to optimize the detection of new variants of *BRCA1/2* genes.

The use of PARP inhibitors in targeted therapy is beneficial for patients with *BRCA1/2* mutations [22, 23, 24]. The Parp1 plays a key role in the repair of single-strand DNA breaks [25]. Recent studies indicate that NGS is increasingly used in routine testing by laboratories. The use of this technology brings benefits to patients in terms of analysis time. NGS is recognized as an efficient method in the detection of both inheritable as well as acquired mutations using DNA from FFPE [26].

**Conclusions**

In our work, we demonstrated that in the diagnosis of hereditary and somatic *BRCA1/BRCA2* mutations, the use of standard procedures may not be sufficient. We shows that NGS with commercial kit for *BRCA1/2* mutants detection from FFPE tumor tissue is fully efficient. The frequency of somatic mutations in ovarian tumors is low (3% − 9%) but their detection may have therapeutic benefits due to the
use of PARP inhibitors [27]. Quick determination of pathogenic variants is important to facilitate specific therapy in addition to the identification of familial predisposition to cancer.

Abbreviations

BC: blood cells; BRCA1: Breast cancer 1, early onset; BRCA2: Breast cancer 2, early onset; CNV: Copy Number Variants; FFPE: formalin fixed paraffin embedded; LGRs: large genetics rearrangements; MLPA: Multiplex Ligation-dependent Probe Amplification; NGS: Next generation sequencing; PARP: poly(adenosine diphosphate)-ribose polymerase; SNVs: single nucleotide variants, VUS: variant as of uncertain significance.

Declarations

Ethics approval and consent to participate

All procedures were approved by the Bioethical Commission in Lublin (approval number 10/2020/KB/VIII). All data were fully anonymized prior to access by the authors.

All procedures were performed in accordance with the Declaration of Helsinki (ethical principles for medical research involving human subjects).

Written informed consent for participation in the study and publication was obtained from the patient described in this case report.

Consent for publication

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Availability of data and materials

The data that support the findings of this study are not publicly available to protect patient privacy, but are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding
Authors' contributions

MO, JW participated in the research design, analysed and interpreted the data, and drafted manuscript, JP, JSS provided samples and compiled clinical data. All authors have read and approve of the final version of the manuscript.

Acknowledgements

We thank all patients who agreed to participate in the current study.

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References


Figures
Representative image of validation of NGS data by Sanger sequencing. Germline mutations were detected in the patient but not in the control. Arrow indicate the position of the mutated nucleotides.
Figure 1

Representative image of validation of NGS data by Sanger sequencing. Germline mutations were detected in the patient but not in the control. Arrow indicate the position of the mutated nucleotides.

Supplementary Files

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