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Analysis of mutations in BRCA1 and BRCA2 genes in tumor tissue from ovarian and breast cancer patients using next generation sequencing (NGS)

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INTRODUCTION

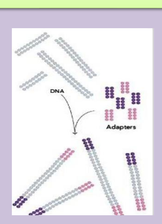
Targeted therapy with PARP inhibitors inhibits growth of breast and ovarian cancer tumor cells harboring a BRCA1 or BRCA 2 mutation. NGS analysis detects both somatic and germline mutations in tumors. Depending on the material (blood/tissue) and selected patient population (e.g. patients with platinum-sensitive ovarian cancer), mutations in the BRCA1/2 genes are found in 11-38% of ovarian and 7-9% breast cancer patients.

AIM

Evaluate the frequency of mutations in the BRCA1 and BRCA2 genes using NGS in a selected group of patients with breast or ovarian cancer.

MATERIALS AND METHODS

Patient eligibility criteria:
- Participated in the National Program for Combating Cancer (between 2006–2015);
- Negative results for the 3-5 most frequent BRCA1/2 mutations;
- Breast (n=45) or ovarian cancer (n=30).



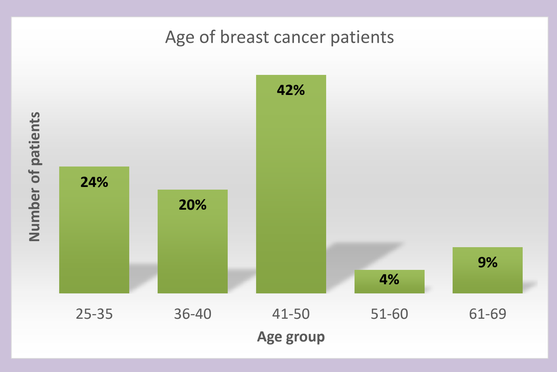
DNA is isolated from FFPE tissue using QIAamp DNA FFPE Tissue Kit(Qiagen). Isolated DNA is qualified and quantified using Real-Time PCR by calculating the ratio of short to long DNA fragments (F-ratio) in a sample using the DNA Fragmentation Quantification Assay (EntroGen).

Libraries are made using BRCA MASTR™ Dx CE-IVD (Multiplicom) for ovarian cancer samples and BRCA Complete™ CE-IVD (EntroGen) for breast cancer samples. Libraries are qualified using the Library Quantification Kit for Illumina® (EntroGen).

Libraries are sequenced on the MiniSeq platform (Illumina). Bioinformatic analysis is performed under the Moncodaneum project (OncoDNA&Walloon Region, Belgium) using the OncoKDM tools developed by OncoDNA.

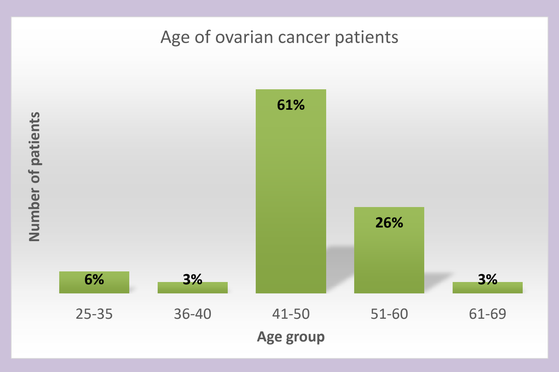
RESULTS

BREAST CANCER



- The average age of diagnosis is 43.
- 44% of patients were diagnosed at age 40 or earlier.
- The youngest patient included in the study was diagnosed at age 25.

OVARIAN CANCER



- The average age of diagnosis is 47.
- 71% of patients were diagnosed at age 40 or earlier.
- The youngest patient included in the study was diagnosed at age 25.

1. Sequencing the BRCA1 and BRCA2 coding regions of DNA isolated from tumor tissues found **4 additional mutations (about 9% more)** in breast cancer patients who were tested negative for BRCA1/2 mutations with the preventative screening program (2006-2015)**.**

Table 1. List of mutations found in breast cancer patients (n=45).

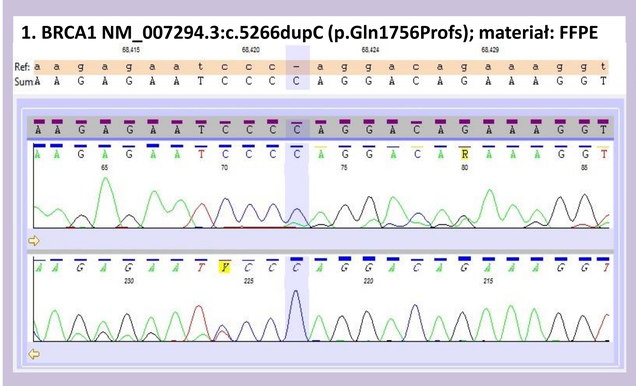
ID	Mutation	Classification	Consequence(s)
1.	BRCA1 NM_007294.3:c.4689C>G (p.Tyr1563Ter)	Pathogenic	Amino acid change, STOP codon (nonsense mutation)
2.	BRCA1 NM_007294.3:c.5123C>A (p.Ala1708Glu)	Pathogenic	Amino acid change (missense mutation)
3.	BRCA1 NM_007294.3:c.1687C>T (p.Gln563Ter)	Pathogenic	Amino acid change, STOP codon (nonsense mutation)
4.	BRCA2 NM_000059.3:c.7758G>A (p.Trp2586Ter)	Pathogenic	Amino acid change, STOP codon (nonsense mutation)

RESULTS

2. Sequencing the BRCA1 and BRCA2 coding regions of DNA isolated from tumor tissues found **7 additional mutations (23% more)** in ovarian cancer patients who were tested negative for BRCA1/2 mutations with the preventative screening program (2006-2015).

Table 2. List of mutations found in ovarian cancer patients (n=30). The first two samples marked with an asterisk (*) are positive controls with known mutations.

ID	Mutation	Classification	Consequence(s)
1.*	BRCA1 NM_007294.3:c.5266dupC (p.Gln1756Profs)	Pathogenic	Change in reading frame
2. *	BRCA1 NM_007294.3:c.181T>G (p.Cys61Gly)	Pathogenic	Amino acid change (missense mutation)
3.	BRCA1 NM_007294.3:c.850C>T (p.Gln284Ter)	Pathogenic	Amino acid change, STOP codon (nonsense mutation)
4.	BRCA1 NM_007294.3:c.406dupA (p.Arg136Lysfs)	Pathogenic	Change in reading frame
5.	BRCA1 NM_007294.3:c.4484+1G>A	Pathogenic	Amino acid change, splice donor site variant
6.	BRCA2 NM_000059.3:c.10095delCinsGAATTATATCT (p.Ser3366Asnfs)	Likely pathogenic/ deleterious benign	Change in reading frame changes STOP codon (nonsense mutation), mRNA splicing (?) Literature: deleterious benign
7.	BRCA2 NM_000059.3:c.6267_6269delGCAinsC (p.Glu2089Aspfs)	Pathogenic	Change in reading frame
8.	BRCA2 NM_000059.3:c.2410G>T (p.Glu804Ter)	Pathogenic	Amino acid change, STOP codon (nonsense mutation)
9.	BRCA2 NM_000059.3:c.6267_6269delGCAinsC (p.Glu2089Aspfs)	Pathogenic	Change in reading frame



3. Mutations are absent in FFPE matched-normal tissue (marked in images above): BRCA1 c.5266dupC (1); BRCA1 c.181T>G (2) (capillary sequencer Applied Biosystems SeqStudio Genetic Analyzer) ; samples are negative for other mutations as well.

CONCLUSIONS

- NGS makes it possible to detect both germline and somatic mutations in the BRCA1 and BRCA2 genes in breast and ovarian cancer tissues.
- Next generation sequencing increases the chance of detecting mutations in the BRCA1 and BRCA2 genes by 23% in patients with ovarian cancer and by 9% in patients with breast cancer compared to the national screening test for the 3-5 most common BRCA mutations in the Polish population.
- Sequencing a matched-normal tissue sample can determine whether a mutation is germline or somatic.
- Older FFPE material resulted in low uniformity of coverage due to DNA fragmentation. Therefore, there is a risk of false negative results in samples with a F-ratio <0.5.
- The detected mutations highlight the importance of bioinformatics tools used to analyze next-generation sequencing results.

Literature:
1. Ratajska M, Krygier M, Stukan M, et al. Mutational analysis of *BRCA1*/2 in a group of 134 consecutive ovarian cancer patients. Novel and recurrent *BRCA1*/2 alterations detected by next generation sequencing. Journal of Applied Genetics. 2015;56(2):193-198.
2. Koczkowska M, Zuk M, Gorczynski A, Ratajska M, Lewandowska M, Biernat W, Limon J, Wasag B. Detection of somatic BRCA1/2 mutations in ovarian cancer - next-generation sequencing analysis of 100 cases.Cancer Med. 2016 Jul;5(7):1640-6.
3. Dann RB, DeLoia JA, Timms KM, Zorn KK, Potter J, Flake DD 2nd, Lanchbury JS, Krivak TC.BRCA1/2 mutations and expression: response to platinum chemotherapy in patients with advanced stage epithelial ovarian cancer. Gynecol Oncol. 2012 Jun;125(3):677-82.

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