Breast and ovarian cancer in Serbia: the importance of mutation detection in hereditary predisposition genes using NGS

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Cancer is consequence of gene mutations

- As a consequence of germline mutations *hereditary cancer* develops.
- As a consequence of somatic (acquired) mutations in somatic cells of particular tissue/organ sporadic cancer develops.
SPORADIC AND HEREDITARY CANCER

Susceptibility to disease is transmitted exclusively from parents to offspring (Mendelian autosomal dominant inheritance) – if one of the parents possess mutation, offspring has 50% inheritance chance
Hereditary breast/ovarian cancer is mainly, but not exclusively related to mutations in highly penetrant BRCA1 and BRCA2 genes

• 5 - 10% of breast and about 20% of ovarian cancers is hereditary

• Hereditary breast and/or ovarian cancer (HBOC), represents significant health problem with a certain social impact
BRCA1/2 – ASSOCIATED CANCERS
LIFE-TIME RISK
EMBRACE, 2013: 978 BRCA1 i 909 BRCA2 positive patients

BREAST CANCER
BRCA1 - 45-84% (EMBRACE, 2013: 60%)
BRCA2 – 45-84% (EMBRACE, 2013: 55%)

MALE BREAST CANCER
BRCA2 – 7%

BILATERAL BREAST CANCER
BRCA1 - 40-60% (EMBRACE, 2013: 83%)
BRCA2- (EMBRACE, 2013: 62%)

OVARIAN CANCER
BRCA1 - 15-45% (EMBRACE, 2013: 59%)
BRCA2 – mutation in OCCR – 20%; other mutation – 11% (EMBRACE, 2013: 16.5%)

ELEVATED RISK FOR OTHER CANCERS (PROSTATE, PANCREAS, MELANOMA, COLON)
BRCA1/2 testing

• Identification of individuals at risk from families with breast/ovarian cancer clustering at risk (affected and healthy)

• Candidate recognition for targeted therapy with PARP inhibitors (ovarian cancer patients)
# HEREDITARY BREAST/OVARIAN CANCER

<table>
<thead>
<tr>
<th></th>
<th>BRCA1</th>
<th>BRCA2</th>
<th>other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast cancer</td>
<td>28%</td>
<td>37%</td>
<td>35% - 70%</td>
</tr>
<tr>
<td>Breast and ovarian</td>
<td>80%</td>
<td>15%</td>
<td>5%</td>
</tr>
<tr>
<td>cancer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ovarian cancer</td>
<td>50-70%</td>
<td>15-25%</td>
<td>5 – 35%</td>
</tr>
</tbody>
</table>
MULTI-GENE TESTING FOR BREAST CANCER HEREDITARY PREDISPOSITION

• **High risk genes** (elevated 5 times in comparison with risk for general population): *BRCA1, BRCA2, TP53, CDH1, PTEN, PALB2, RAD51C, RAD51D, MLH1, MSH2, MSH6, PMS2

• **Moderate risk genes** (elevated 1.5-5 times in comparison with risk for general population): *ATM, BRIP, CHEK2* (25-37%)

• **Low risk genes** (elevated below 1.5 times in comparison with risk for general population): *FGFR2, MAP3K1, CASP8*

• **NCCN Clinical Practice Guidelines in Oncology: Genetic/Familial High-Risk Assessment: Breast and Ovarian v 1.2018** – panel consisted of 19 genes
RECOMMENDATION FOR HEREDITARY BREAST/OVARIAN CANCER DETECTION IS MULTI-GENE

• **NGS technology** enables gene panel testing in one step due to higher sensitivity, increased rate of mutation detection and reduced costs of testing.

• **Multi-gene testing** enables identification of mutations in genes other than *BRCA1/2*-even **17% of **BRCA1/2** negative families with high risk for breast cancer showed mutation in genes other than **BRCA1/2**.**
HEREDITARY BREAST/OVARIAN CANCER

• **Gene panel** (19 genes) recommended by National Comprehensive Cancer Network (NCCN):
  • ATM, BRCA1, BRCA2, BRIP1, CDH1, CHEK2
  • genes responsible for Lynch syndrome (MSH2, MLH1, MSH6, PMS2, EPCAM)
  • NBN, NF1, PALB2, PTEN, RAD51C, RAD51D, STK11, TP53
Testing in hereditary breast/ovarian cancer


Multi-Gene (NGS) Panels
Testing for hereditary breast/ovarian cancer

The BRCA Hereditary Cancer MASTR™ Plus

26 genes

The BRCA Hereditary Cancer MASTR™ Plus

30 genes

Illumina TruSight® Cancer Panel

96 genes

TruSight Cancer 94-Gene pre-disposition Panel for detecting Germine mutations

AIP  BUB1B  DDB2  EXT2  FANCL  MEN1  PALB2  RB1  SLX4  WRN
ALK  CDC73  Dicer1  EZH2  FANCM  MET  PH0X2B  RECG4  SMAD4  WT1
APC  CDH1  DIS3L2  FANCIA  FH  MLH1  PMS1  RET  SMARC1  XPA
ATM  C0K4  EGFR  FANCB  FLCN  MSH2  PMS2  RHBDF2  STK11  XPC
BAP1  C0KN1C  EPCAM  FANCC  GATA2  MSH6  PRF1  RUNX1  SUFU
BLM  C0KN2A  ERCC2  FANCID  GPC3  MUTYH  PRKAR1A  SBDS  TMEM127
BMP1R  CEBPA  ERCC3  FANCJ  HNF1A  NBN  PTCH1  SODH1  TP53
BRCA1  CEPP7  ERCC4  FANC1  HRAS  NF1  PTEN  SDHB  TSC1
BRCA2  CHEK2  ERCC5  FANG2  KIT  NF2  RAD51C  SDHC  TSC2
BRIP1  CYLD  EXT1  FANCI  MAX  NSD1  RAD51D  SDHD  VHL
<table>
<thead>
<tr>
<th>Class</th>
<th>Description</th>
<th>Probability of pathogenicity</th>
<th>Clinical predictive testing of family members</th>
<th>Clinical recommendation for mutation carriers</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>Pathogenic</td>
<td>&gt;0,99</td>
<td>yes</td>
<td>Complete clinical follow-up</td>
</tr>
<tr>
<td>4</td>
<td>Likely pathogenic</td>
<td>0,95-0,99</td>
<td>yes</td>
<td>Complete clinical follow-up</td>
</tr>
<tr>
<td>3</td>
<td>Unknown (VUS)</td>
<td>0,05-0,949</td>
<td>no</td>
<td>Not crucial for risk determination</td>
</tr>
<tr>
<td>2</td>
<td>Likely benign</td>
<td>0,001-0,049</td>
<td>no</td>
<td>Family history only- based estimation of risk</td>
</tr>
<tr>
<td>1</td>
<td>Benign</td>
<td>&lt;0,001</td>
<td>no</td>
<td>Family history only- based estimation of risk</td>
</tr>
</tbody>
</table>
BRCA1/2 testing results:

- Harmful (pathogenic) mutation with known clinical significance
- Genetic variant with unknown significance (VUS)
- Polymorphism – benign variant
Testing in hereditary breast/ovarian cancer

Insufficient evidence to determine if the variant is associated with an increased cancer risk
Germline mutation detection - HBOC

Jun 2016 - May 2018

Totally tested by NGS n= 264

Additionally tested for family mutations by Sanger n= 25

NGS results: n= 264

11 (4.2%) BRCA1 MUT
28 (10.7%) BRCA2 MUT
225 (85.1%) WT

Ovarian cancer (n=123)

100 (81.4%) WT
18 (14.6%) BRCA1 MUT
5 (4%) BRCA2 MUT

Breast cancer (n=113)

98 (86.7%) WT
9 (7.9%) BRCA1 MUT
6 (5.4%) BRCA2 MUT

Healthy (n=28)

27 (96.4%) WT
1 (3.6%) BRCA1 MUT

8pts/23 reported family history of breast/ovarian cancers
NGS results, breast cancer n=113

- **BRCA1**: 9
- **BRCA2**: 6
- **PALB2**: 7
- **ATM**: 3
- **CHEK2**: 3
- **RET**: 2
- **TP53**: 2
- **CDH1 VUS**: 3
- **PRF1 VUS**: 3
- **RAD51D VUS**: 2
- **MSH2 VUS**: 2
- **NBN VUS**: 1
- **TP53 VUS**: 1
- **PMS2 VUS**: 1
- **BRCA1 VUS**: 1
- **BRCA2 VUS**: 1
- **PALB2 VUS**: 1
- **CDH1 VUS**: 1
- **RET VUS**: 1
- **TP53 VUS**: 1
- **PRF1 VUS**: 1
- **RAD51D VUS**: 1
- **MSH2 VUS**: 1
- **NBN VUS**: 1
- **TP53 VUS**: 1
- **PMS2 VUS**: 1
Germline mutation detection - HBOC

NGS results, ovarian cancer n=123

- BRCA1
- BRCA2
- CHEK2
- RET
- RAD51C
- BRIP1
- NBN
- BRCA2 VUS
- PALB2 VUS
- BRIP1 VUS
- ATM VUS
- RET VUS
- PRF1 VUS
- RAD51D VUS
- MSH6 VUS
- MSH2 VUS
- NBN VUS
- PMS2 VUS
Germline mutation detection - HBOC

NGS results, healthy patients $n=28$

- **BRCA1**: 1
- **CHEK2**: 2
- **BRCA2 VUS**: 3
- **PALB2 VUS**: 1
- **ATM VUS**: 2
- **NBN VUS**: 2
- **MSH2 VUS**: 1
- **BRCA1 VUS**: 1
- **CHEK2 VUS**: 2
- **MSH2 VUS**: 1
- **BRCA2 VUS**: 1
Germline mutation detection - HBOC

Totally tested by NGS n= 264

Additionally tested for family mutations by Sanger n= 25

- BRCA1 MUT: 9
- BRCA2 MUT: 6
- PALB2 MUT: 3
- CHEK2 MUT: 1
- ATM MUT: 1
- WAITING RESULT: 1

WT for family mutation: 4

Jun 2016 - May 2018
Detekcija germinativnih mutacija u karcinomu dojke i jajnika - IORS

BRCA full scheme

• Assessment of genotyping, and **biological and clinical interpretation**.
  • For each case, participants are expected to return a clinical report which includes a **complete (biological and clinical) interpretation of the results.**
Germline mutation detection in breast/ovarian cancer families in Serbia

**CHEK2 gene** - a cell cycle checkpoint regulator and putative tumor suppressor

<table>
<thead>
<tr>
<th>CANCER TYPE</th>
<th>AGE RANGE</th>
<th>CANCER RISK</th>
<th>RISK FOR GENERAL POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male Breast</td>
<td>To age 80</td>
<td>0.4%-1%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Colorectal</td>
<td>To age 80</td>
<td>Possibly elevated</td>
<td>3.4%</td>
</tr>
</tbody>
</table>

**NM_007194.3(CHEK2):c.444+1G>T**

- **Clinical significance:** Pathogenic/Likely pathogenic
- **Last evaluated:** Jan 5, 2004
- **Number of submission(s):** 1
- **Condition(s):** Familial cancer of breast

- **up to 1% male breast cancer risk, elevated colorectal cancer risk**
Germline mutation detection in breast/ovarian cancer families in Serbia

**PMS1** - DNA mismatch repair gene, out of NCCN19 gene panel

c.1081_1085delAGTAA, p.Ser361AsnfsTer9 - Frameshift - VUS?

Not reported previously
Unclassified
Unknown clinical significance
Germline mutation detection in breast and ovarian cancers in Serbia

**ERCC3** - nucleotide excision repair gene, out of NCCN19 gene panel

**NM_000122.1(ERCC3):c.325C>T (p.Arg109Ter)**

- Variation ID: 265515
- Review status: criteria provided, single submitter

**Clinical significance:** Likely pathogenic

**Last evaluated:** Dec 24, 2015

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A Recurrent ERCC3 Truncating Mutation Confers Moderate Risk for Breast Cancer.

Vijai L1,2, Topka S1,2, Villano D1,2, Ravichandran V1,2, Maxwell KN3,4, Maria A1,2, Thomas J1,2, Gaddam P1,4, Lincoln S1,4, Karzaz S1,2, Wenz G3, Carmi S5, Schrader K5, Hart SN7, Lipkin SM8, Neuhausen SL9, Walsh M1,4,10, Zhang L11, Lejbkowicz F12, Rennert G12, Stadier ZK1,4,8, Robson M1,4,8, Weitzel JN13, Roehmchek C3,14,16, Daily M15,16,17, Couch F17,18, Nathanson K1,3,14,16, Norton L1, Rennert G12, Offit K19,2,4,8
Germline mutation detection in breast/ovarian cancer families in Serbia

ATM- DNA-damage response gene

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<thead>
<tr>
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<th>AGE RANGE</th>
<th>CANCER RISK</th>
<th>RISK FOR GENERAL POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female Breast</td>
<td>To age 50(^3)</td>
<td>Up to 9%</td>
<td>1.9%</td>
</tr>
<tr>
<td>Female Breast</td>
<td>To age 80(^1,2,3)</td>
<td>17%-52%</td>
<td>10.2%</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>To age 80(^4)</td>
<td>Elevated risk</td>
<td>1%</td>
</tr>
</tbody>
</table>

(\(ATM\)): c.8983C>A, p.Leu2995Ile

Pathogenic mutation carriers- up to 52% breast cancer risk, elevated pancreatic cancer risk
Germline mutation detection in breast/ovarian cancer families in Serbia

**MLH1**- tumor suppressor gene involved in DNA mismatch repair

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<th>AGE RANGE</th>
<th>CANCER RISK</th>
<th>RISK FOR GENERAL POPULATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal</td>
<td>To age 70(^{1,6})</td>
<td>52%-82%</td>
<td>1.9%</td>
</tr>
<tr>
<td>Endometrial</td>
<td>To age 70(^{1,6})</td>
<td>25%-60%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Overall cancer risk (Lynch cancers)</td>
<td>Increased risk</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Ovarian</td>
<td>To age 70(^{1,6})</td>
<td>4%-12%</td>
<td>0.7%</td>
</tr>
<tr>
<td>Gastric</td>
<td>To age 70(^{1,6})</td>
<td>6%-13%</td>
<td>0.3%</td>
</tr>
<tr>
<td>Small Bowel</td>
<td>To age 70(^{1,6})</td>
<td>3%-6%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Urinary Tract</td>
<td>To age 70(^{1,6})</td>
<td>1%-7%</td>
<td>&lt;1.0%</td>
</tr>
<tr>
<td>Pancreatic</td>
<td>To age 70(^{1,6})</td>
<td>1%-6%</td>
<td>0.5%</td>
</tr>
<tr>
<td>Hepatobiliary Tract</td>
<td>To age 70(^{1,6})</td>
<td>1.4%-4%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Central Nervous System</td>
<td>To age 70(^{1,6})</td>
<td>1%-3%</td>
<td>0.4%</td>
</tr>
<tr>
<td>Sebaceous Neoplasms</td>
<td>To age 70(^{1,6})</td>
<td>1%-9%</td>
<td>&lt;1.0%</td>
</tr>
</tbody>
</table>

**NM_000249.3(MLH1): c.1451A>G (p.Asp484Gly)**

**Variation ID:** 232486
**Review status:** criteria provided, single submitter

**Interpretation:** Uncertain significance

**Clinical significance:**
- Last evaluated: Apr 11, 2016
- Number of submission(s): 1
- Condition(s): Hereditary cancer-predisposing syndrome [MacGen]


Increased ovarian cancer risk for pathogenic mutation carriers
Germline mutation detection in breast/ovarian cancer families in Serbia

**BRCA2, NBN** - DNA double-strand break repair

**VUS (BRCA2):** c.2944A>C (p.Ile982Leu)
- Increased breast (43%-84%) and ovarian (16.5%-27%) cancer risk for pathogenic mutation carriers

**VUS (NBN):** c.511A>G (p.Ile171Val)
- Increased breast (up to 30%) cancer risk for pathogenic mutation carriers
Germline mutation detection in breast/ovarian cancer families in Serbia

PALB2 - homologous recombination repair gene

**VUS** *(PALB2)*: c.2922G>T (p.Lys974Asn)

Increased breast (17-58%) cancer risk and elevated pancreatic cancer risk for pathogenic mutation carriers.
Conclusions

• Identification of germline mutations in other genes by NGS contributes to better understanding of hereditary predisposition

• Future investigation has to define risk for particular gene and particular pathogenic mutation in HBOC genes

• In the mean time, the surveillance protocol has to be carefully customized according to personnel and family history and patient genetic characteristics