Contemporary vs. Traditional DNA-Based Mutation Testing to Detect Hereditary Breast and Ovarian Cancer Syndrome (HBOC) in Women: A Meta Narrative Review

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BACKGROUND

- Breast cancer one of the most common cancers in women
- Inherited genetic mutations in BRCA genes 1 & 2 (BRCA 1/2) cause cancer if wild-type allele is inactive
  → Hereditary Breast and Ovarian Cancer Syndrome
- HBOC caused by an autosomal dominant mutation genetically passed down
  - Predisposes individuals to an increased risk of developing early onset breast and ovarian cancers
### Molecular In My Pocket™

**ONCOLOGY: Breast Cancer**

<table>
<thead>
<tr>
<th>Tumor Group</th>
<th>Gene/Biomarker</th>
<th>Alterations</th>
<th>Indications</th>
<th>Result Interpretation/Significance</th>
<th>Assay Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasive Ductal/Lobular Carcinoma</td>
<td><strong>BRCA1/2</strong></td>
<td>Inactivating variants</td>
<td>Germline testing, therapeutic</td>
<td>Hereditary breast cancer, predicts response to PARP-inhibitors</td>
<td>CMA, MLPA, NGS, PCR, Sanger</td>
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<tr>
<td></td>
<td><strong>CDH1</strong></td>
<td>Inactivating variants</td>
<td>Germline testing, diagnostic</td>
<td>Inactivated in lobular carcinomas (in-situ and invasive), germline pathogenic variants associated with susceptibility to ILC and hereditary diffuse gastric cancer</td>
<td>CMA, IHC (E-cadherin), MLPA, NGS, PCR, Sanger</td>
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<tr>
<td></td>
<td><strong>ERBB2</strong></td>
<td>Amplification, activating variants</td>
<td>Therapeutic</td>
<td>Molecular/intrinsic subtype classification, predicts response to anti-HER2 therapies</td>
<td>FISH, IHC, NGS</td>
</tr>
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<td></td>
<td><strong>ER/PR</strong></td>
<td>Increased expression</td>
<td>Prognostic, therapeutic</td>
<td>Molecular/intrinsic subtype classification, predicts response to endocrine therapy</td>
<td>IHC</td>
</tr>
<tr>
<td></td>
<td><strong>IDH2</strong></td>
<td>p.Arg172 hotspot variants</td>
<td>Diagnostic, possibly therapeutic in the future</td>
<td>Associated with tall cell carcinoma with reversed polarity</td>
<td>NGS, PCR, Sanger</td>
</tr>
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<td></td>
<td><strong>PIK3CA</strong></td>
<td>Activating variants</td>
<td>Therapeutic</td>
<td>Predicts response to alpelisib; enriched in luminal A tumors</td>
<td>NGS, PCR, Sanger</td>
</tr>
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<td></td>
<td><strong>PTEN</strong></td>
<td>Inactivating variants</td>
<td>Germline testing</td>
<td>Cowden syndrome</td>
<td>CMA, MLPA, NGS, PCR, Sanger</td>
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<td></td>
<td><strong>STK11</strong></td>
<td>Inactivating variants</td>
<td>Germline testing</td>
<td>Peutz-Jeghers syndrome</td>
<td>CMA, MLPA, NGS, PCR, Sanger</td>
</tr>
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<td></td>
<td><strong>TP53</strong></td>
<td>Inactivating variants</td>
<td>Germline testing</td>
<td>Li-Fraumeni syndrome, enriched in basal-like tumors</td>
<td>CMA, MLPA, NGS, PCR, Sanger</td>
</tr>
<tr>
<td>Salivary Gland-Type Neoplasms</td>
<td><strong>CRTC1::MAML2</strong></td>
<td>Fusion</td>
<td>Diagnostic</td>
<td>Associated with mucoepidermoid carcinoma</td>
<td>FISH, NGS, RT-PCR</td>
</tr>
<tr>
<td></td>
<td><strong>CRTC3::MAML2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td><strong>ETV6::NTRK3</strong></td>
<td>Fusion</td>
<td>Diagnostic, therapeutic</td>
<td>Associated with secretory carcinoma, predicts response to larotrectinib and entrectinib</td>
<td>FISH, IHC (pan-TRK), NGS, RT-PCR</td>
</tr>
<tr>
<td></td>
<td><strong>HMGA2 or PLAG1</strong></td>
<td>Fusion</td>
<td>Diagnostic</td>
<td>Associated with pleomorphic adenoma</td>
<td>FISH, IHC (HMGA2, PLAG1), NGS, RT-PCR</td>
</tr>
<tr>
<td></td>
<td><strong>MYB::NFB and MYBL1::NFB</strong></td>
<td>Fusion</td>
<td>Diagnostic</td>
<td>Associated with adenoid cystic carcinoma</td>
<td>FISH, IHC/ISH (MYB), NGS, RT-PCR</td>
</tr>
</tbody>
</table>
SIGNIFICANCE

• Contemporary DNA testing using multigene panels has the potential to identify harmful mutations not only in BRCA genes but in other potentially harmful variants that predispose to HBOC.

• It is important for healthcare professionals to recognize susceptible patients early on.

• Contemporary technologies in multigene paneling are improving healthcare by streamlining diagnostic procedures.

• Next-Generation Sequencing is an efficient alternative to the traditional method of Sanger Sequencing.

• These new genetic testing methods may have a positive impact on those that are high risk for HBOC, allowing patients greater options and time to implement preventative measures if needed.
RESEARCH QUESTION

Does the use of multi-gene panel testing increase the probability for detection of HBOC-associated mutations compared to testing only BRCA1 and BRCA2?
HYPOTHESIS

Women who undergo multi-gene panel testing will have a higher detection rate for cancer-predisposing mutations compared to women who receive testing focused only on BRCA1 and BRCA2.
**Inclusion Criteria**
- Relevance: documents should directly address genetic testing methods for HBOC in women
- Recent publications: preference should be given to recent publications (5-year range).
- Diverse methodologies: experimental, observational, systematic, and meta-synthesis studies allow for a comprehensive examination from various perspectives.

**Key Words:** Hereditary breast and ovarian cancer syndrome (HBOC), BRCA1, BRCA2, multi-gene panel testing, Next-generation sequencing, Sanger sequencing

**Exclusion Criteria:**
- Studies that do not contribute relevant narratives or perspectives to the research question.
- Studies that focus on other types of cancers with no mention of breast cancer, ovarian cancer, or HBOC.
- Literature that lacks credibility, such as unreliable sources or publications with methodological flaws.
- Literature is not from a reputable and reliable source.
- Duplicate publications or multiple reports of the same study.
- Studies published outside the defined 5-year time frame
- Literature written in languages that are not accessible for translation or interpretation.
# Sample Processing Criteria

## Sanger Sequencing

<table>
<thead>
<tr>
<th>Criteria</th>
<th>Sanger sequencing</th>
<th>Next-Generation Sequencing (NGS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Accuracy</td>
<td>99.9%</td>
<td>99 – 99.9%</td>
</tr>
<tr>
<td>Sample Limit</td>
<td>Up to 20 samples</td>
<td>More than 20 samples</td>
</tr>
<tr>
<td>Speed (&lt;20 samples)</td>
<td>Fast</td>
<td>Slow</td>
</tr>
<tr>
<td>Speed (&gt;20 samples)</td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>15 – 20%</td>
<td>1%</td>
</tr>
<tr>
<td>Sample coverage</td>
<td>1 read/sample (300 – 850 bp)</td>
<td>Billions of Reads/Samples (up to 16Tb )</td>
</tr>
</tbody>
</table>

### Pros:
- Fast
- Accurate
- Cost-effective for < 20 samples

### Cons:
- Maximum of only 20 samples
- 1 read/sample

## Next-Generation Sequencing (NGS)

### Pros:
- Fast turnaround time
- Accurate
- Can test more than 20 samples
- High sensitivity allows for detection of low-frequency variants

### Cons:
- Expensive
- Time-consuming for 1-20 targets
## COSTS OF SEQUENCING

<table>
<thead>
<tr>
<th>Sequencing Method</th>
<th>Platform</th>
<th>Target size (bp)</th>
<th>Cost (per sample, USD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Next-Generation Sequencing</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WGS</td>
<td>~ 3x10^9</td>
<td>$1000 - $3000</td>
<td></td>
</tr>
<tr>
<td>WES</td>
<td>~ 6x10^7</td>
<td>$500 - $2000</td>
<td></td>
</tr>
<tr>
<td>TS</td>
<td>~ 1x10^5 – 1x10^7</td>
<td>$300 - $1000</td>
<td></td>
</tr>
<tr>
<td>Sanger Sequencing</td>
<td></td>
<td>~ 3x10^2 – 1x10^3</td>
<td>$30</td>
</tr>
</tbody>
</table>

Comparison the costs between genetics sequencing methods: NGS (Next Generation Sequencing), WGS (Whole Genome Sequencing), WES (Whole Exome Sequencing), TS (Targeted Sequencing) and Sanger sequencing.
STRENGTHS AND LIMITATIONS

STRENGTHS
• Recent studies (within 5-year timeframe: 2019-2024)
• Appropriate statistical methods were used
• Able to combine large and complex research

LIMITATIONS
• Meta-narrative based on a limited number of sources and information
• Secondary research
• Overused data
• Increased risk of bias
The future of HBOC diagnosis and treatment lies in the field of Genetic Counseling.

By implementing the use of expansive tests such as multi-gene or NGS, the specific genes that are causing HBOC in patients would be detected early on.

Doctors and genetic counselors will be able to accurately predict:

- The effectiveness of treatments
- The course and risks of metastasis
- Track inheritable qualities that could be present in an offspring

These advancements could potentially lessen the severity of HBOC over time.
CONCLUSION

- Our findings **support** the hypothesis because detection rates for HBOC tend to increase when multigene panels are used compared to single gene testing; “significant differences were observed regarding variant pathogenicity (p<0.0001) … the multigene panel was able to significantly resolve more high-risk patients” because of the vast panel of genes tested (Molina-Zaya, et al., 2022).

- Study findings support the recommendation for more widespread use of multigene panel genetic tests.

- A change to mainly multigene panel tests is likely to be seen in the future. The high cost of new genetic methods can be justified by the better detection outcomes in diagnosis of HBOC-causing germline mutations.
REFERENCES


REFERENCES CONT…


