Traditional vs. next-generation panel testing of hereditary breast and ovarian cancer genes in a large clinical population

Stephen E. Lincoln
Mutations in 12 genes for inherited ovarian, fallopian tube, and peritoneal carcinoma identified by massively parallel sequencing

Tom Walsh\textsuperscript{a}, Silvia Casadei\textsuperscript{a}, Ming K. Lee\textsuperscript{a}, Christopher C. Pennil\textsuperscript{b}, Alex S. Nord\textsuperscript{a}, Anne M. Thornton\textsuperscript{a}, Wendy Roeb\textsuperscript{a}, Kathy J. Agnew\textsuperscript{b}, Sunday M. Stray\textsuperscript{a}, Anneka Wickramanayake\textsuperscript{b}, Barbara Norquist\textsuperscript{b}, Kathryn P. Pennington\textsuperscript{b}, Rochelle L. Garcia\textsuperscript{c}, Mary-Claire King\textsuperscript{a,1}, and Elizabeth M. Swisher\textsuperscript{a,b,1}

• 360 patients
• Ovarian, fallopian or peritoneal cancer
• Unselected for age of onset or family history
• 17.5% BRCA1/BRCA2 positive
• 6.1% Non-BRCA positive

Invitae-Stanford pilot study

- 198 patients met NCCN guidelines for BRCA testing
  - Age of onset, family history, Ashkenazi background, or other factors
  - Most with breast cancer
  - Some with ovarian cancer (Fx also pancreas, prostate, colon)
  - Enriched for BRCA positives to aid in sensitivity analysis

- 141 BRCA negative

- 10 of 141 (7.1%) non-BRCA positive
  - Reported results, management changes for some of these patients
  - Additional 5 of 141 (3.6%) MUTYH heterozygotes

Case study
Cancer

Frequency of Mutations in Individuals With Breast Cancer Referred for BRCA1 and BRCA2 Testing Using Next-Generation Sequencing With a 25-Gene Panel

Nadine Tung, MD1,2; Chiara Battelli, MD1; Brian Allen, MS3; Rajesh Kaldete, MS3; Satish Bhatnagar, PhD4; Karla Bowles, PhD5; Kirsten Timms, PhD6; Judy E. Garber, MD7; Christina Harold, MD1,2; Leif Ellisen, MD, PhD2,8; Jill Krejdosky, MS9; Kim DeLeonardis, MS9; Kristin Sedgwick, MS5; Kathleen Soltis, MA9; Kathleen Soltis, MA9; Benjamin Roa, PhD5.

ORIGINAL RESEARCH ARTICLE

Utilization of multigene panels in hereditary cancer predisposition testing: analysis of more than 2,000 patients

Holly LaDuca, MS1,2, AJ Stuenkel, MS1, Jill S. Dolinsky, MS1, Steven Keiles, MS1, Stephany Tandy, MS1, Tina Pesaran, MA, MS1, Elaine Chen, MS1, Chia-Ling Gau, PhD1, Erika Palmaer, BA1, Kamelia Shoapour, BS1, Divya Shah, MS2, Virginia Speare, PhD1, Stephanie Gandomi, MS1 and Elizabeth Chao, MD1,3

ARTICLE

Next-generation sequencing for the diagnosis of hereditary breast and ovarian cancer using genomic capture targeting multiple candidate genes

Laurent Castérin1,2, Sophie Krieger1,2,3, Antoine Rousselin1, Angéline Legros1, Jean-Jacques Baumann1, Olivia Bruet1, Baptiste Braault1, Robin Fouillet1, Nicolas Goardon1, Olivier Letac1, Stéphanie Baert-Desurmont2,4, Julie Tinat2,4, Odile Bera5, Catherine Dugast6, Pascaline Berthet7, Florence Polycarpe7, Valérie Layet6, Agnes Hardouin1,2, Thierry Frébourg2,4,9 and Dominique Vaur4,1,2
Prevalence of mutations in a panel of breast cancer susceptibility genes in BRCA1/2-negative patients with early-onset breast cancer

Inherited Mutations in 17 Breast Cancer Susceptibility Genes Among a Large Triple-Negative Breast Cancer Cohort Unselected for Family History of Breast Cancer

Questions about clinical multi-gene tests

1. Can next-generation sequencing (NGS)-based panel tests replace traditional tests in terms of sensitivity, specificity, and completeness?
   - Including copy-number changes?
   - Complex variants?

2. Can laboratories without large, proprietary databases interpret variants in ways comparable to a lab that uses one?
   - What is the impact of the new ACMG guidelines for interpretation of sequence variants?

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Silver standards

Sanger sequencing and capillary electrophoresis

- Are certainly not perfect, but...
- Can detect some variants that are hard for next-generation sequencing
  - Larger insertions/deletions, homopolymer-associated, complex changes

Microarrays, qPCR, MLPA for CNVs

- Work well when probe binding sites are not disrupted
## 29 gene hereditary cancer panel

<table>
<thead>
<tr>
<th>Sub-panel</th>
<th>Genes</th>
<th>Total</th>
<th>Gene names</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1/BRCA2</td>
<td>2</td>
<td>2</td>
<td>BRCA1, BRCA2</td>
</tr>
<tr>
<td>Other high-risk breast/ovarian</td>
<td>4</td>
<td>6</td>
<td>CDH1, PTEN, STK11, TP53</td>
</tr>
<tr>
<td>moderate-risk breast/ovarian</td>
<td>6</td>
<td>12</td>
<td>ATM, BRIP1, CHEK2, NBN, PALB2, RAD51C</td>
</tr>
<tr>
<td>Lynch Syndrome</td>
<td>5</td>
<td>17</td>
<td>EPCAM, MLH1, MSH2, MSH6, PMS2</td>
</tr>
<tr>
<td>Other hereditary cancer syndromes</td>
<td>11</td>
<td>28</td>
<td>APC, BMPR1A, SMAD4, CDK4, CDKN2A, PALLD, MET, MEN1, RET, PTCH1, VHL</td>
</tr>
<tr>
<td>MUTYH</td>
<td>1</td>
<td>29</td>
<td>MUTYH</td>
</tr>
</tbody>
</table>
# Expanded study

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Description</th>
<th>Previous testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prospective clinical</td>
<td>735</td>
<td>Prospective clinical cases</td>
<td>Clinical testing for BRCA1/BRCA2; occasionally on other genes (depending on case) using traditional methods</td>
</tr>
<tr>
<td>High-risk clinical (total 327)</td>
<td>209</td>
<td>Retrospective cases from a clinical biobank, generally containing higher-risk individuals</td>
<td>Clinical single-site testing</td>
</tr>
<tr>
<td></td>
<td>118</td>
<td>Cases referred due to known pathogenic variant in family</td>
<td></td>
</tr>
<tr>
<td>Reference samples</td>
<td>36</td>
<td>Reference samples from public biobanks</td>
<td>Samples carrying known pathogenic variants</td>
</tr>
<tr>
<td>Well-characterized genomes (WCGs)</td>
<td>7</td>
<td>Reference samples from public biobanks with high-quality whole genome sequencing (WGS) data</td>
<td>Variants in 29 cancer genes extracted from WGS data; most benign</td>
</tr>
<tr>
<td>Total</td>
<td>1105</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Analytic concordance vs. traditional methods

## Previous testing; independent confirmation (combined)

<table>
<thead>
<tr>
<th></th>
<th>Variant present</th>
<th>Variant not present</th>
<th>Conclusion</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Variant detected</strong></td>
<td>750 true positives</td>
<td>0 false positives</td>
<td>100% specificity</td>
</tr>
<tr>
<td><strong>Variant not detected</strong></td>
<td>0 false negatives</td>
<td>Sequence: 15.0 million true negative base-pairs</td>
<td>100% sensitivity</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CNV: 22.2 thousand true negative exons</td>
<td></td>
</tr>
</tbody>
</table>
### Variants selected for analytic validation study

<table>
<thead>
<tr>
<th>Type</th>
<th>Variants</th>
<th>Details</th>
</tr>
</thead>
<tbody>
<tr>
<td>Single nucleotide variants (SNVs)</td>
<td>549</td>
<td></td>
</tr>
<tr>
<td>Sequence deletions &lt;10 base-pairs</td>
<td>125</td>
<td></td>
</tr>
<tr>
<td>Sequence insertions &lt;5 base-pairs</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Sequence insertions ≥5 base-pairs</td>
<td>4</td>
<td>24, 5 bp</td>
</tr>
<tr>
<td>Sequence deletions ≥10 base-pairs</td>
<td>9</td>
<td>126, 40, 19, 15, 11 bp</td>
</tr>
<tr>
<td>Complex variants</td>
<td>6</td>
<td>Delins, haplotypes, homopolymer-associated¹</td>
</tr>
<tr>
<td>Single exon deletions</td>
<td>9</td>
<td>BRCA1, BRCA2, MSH2, PMS2</td>
</tr>
<tr>
<td>Single exon duplications</td>
<td>4</td>
<td>BRCA1, MLH1</td>
</tr>
<tr>
<td>Deletions of multiple exons or whole gene</td>
<td>10</td>
<td>BRCA1, MSH2, RAD51C</td>
</tr>
<tr>
<td>Duplications of multiple exons or whole gene</td>
<td>6</td>
<td>BRCA1, BRCA2, NBN, SMAD4</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>750</strong></td>
<td></td>
</tr>
</tbody>
</table>

Some published validation studies have few, if any, examples of these relatively challenging classes of variation.²,³

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¹ MSH2:c.942+3A>T  
A significant fraction of the pathogenic variants in clinical cases are challenging for next-generation sequencing.

Pathogenic and likely pathogenic variants (n=260) among the clinical cases (n=1062) by variant type
How to get to 100% sensitivity and specificity with next-generation sequencing?

No single answer. No single approach.

Biochemistry

- **Multiple target enrichment approaches** together
  - Fill in poorly covered regions.
- Optimize protocols to make raw data **highly callable**
  - Even and reproducible coverage
  - Measure remaining variability with in-batch controls.

Bioinformatics

- **Good aligner** (= computationally expensive)
- **Multiple callers**, each good at different things
**Bioinformatics pipeline**

*The whole is greater than the sum of the parts.*

- **NovoAlign or BWA-MEM**
  - GATK Unified Genotyper
  - Freebayes
  - Read-depth analysis
  - Split-read analysis
  - CoalGen

**Outputs:**
- SNVs, indels
- Del/dup events
- Homopolymer-associated variants
Next-generation sequencing read-depth profiles

- **Read depth varies** between targets, due to both the targeting chemistry and next-generation sequencing itself.

- However, **relative coverage is reproducible**, allowing copy number changes to be detected by a comparison of a patient’s result to a panel of **in-batch controls**.
Example: BRCA1 exon 19 deletion

These are *raw unprocessed data*. After processing, this signal is even more clear.
Split-read signals in next-generation sequencing

Example of a deletion

Reference genome

Deleted region

NGS read
BRCA2: c.9203del126

Split-read signal at 5’ end of deletion

Split-read signal at 3’ end of deletion

Exon target
BRCA1: c.1175_1214del40

- Deletion mapped correctly in a fraction of reads
- Split-read signature in additional reads
BRCA2 c.156_insAlu

Split-read signal of Alu sequence
SMAD4 whole-gene duplication (CN=3)

- Novel pseudogene, no introns
- Exon targets
- Split-read signal at edge of exon in one-third of reads

Same

Same

Same
MSH2:c.943+3T>C

25bp poly-A
Questions about clinical multi-gene tests

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### Interpretation concordance

#### Previous BRCA1/BRCA2 testing

<table>
<thead>
<tr>
<th></th>
<th>Positive</th>
<th>Uncertain</th>
<th>Negative</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>188</td>
<td></td>
<td></td>
<td>188 (19.3%)</td>
</tr>
<tr>
<td>Uncertain</td>
<td>2</td>
<td>30</td>
<td>8</td>
<td>787 (80.7%)</td>
</tr>
<tr>
<td>Negative</td>
<td></td>
<td>1</td>
<td>746</td>
<td>785 (80.5%)</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>190 (19.5%)</td>
<td>785 (80.5%)</td>
<td></td>
<td>975 (100%)</td>
</tr>
</tbody>
</table>

**Net result concordance:** 99.8% (973/975 cases)

**Uncertain reports:** 4.1% (40/975 cases) vs. 3.2% (31/975 cases)
Accumulation of new variants requiring interpretation

Our current rate: 0.3–0.6 variants per patient
Questions about clinical multi-gene tests

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Pathogenic variants in 1105 individuals

<table>
<thead>
<tr>
<th>Gene</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>119</td>
</tr>
<tr>
<td>BRCA2</td>
<td>79</td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
</tr>
<tr>
<td>TP53</td>
<td>2</td>
</tr>
<tr>
<td>CDH1</td>
<td>4</td>
</tr>
<tr>
<td>STK11</td>
<td></td>
</tr>
<tr>
<td>PALB2</td>
<td>5</td>
</tr>
<tr>
<td>CHEK2</td>
<td>5</td>
</tr>
<tr>
<td>ATM</td>
<td>9</td>
</tr>
<tr>
<td>BRIP1</td>
<td>1</td>
</tr>
<tr>
<td>RAD51C</td>
<td>3</td>
</tr>
<tr>
<td>NBN</td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>1</td>
</tr>
<tr>
<td>MSH2</td>
<td>2</td>
</tr>
<tr>
<td>MSH6</td>
<td>2</td>
</tr>
<tr>
<td>EPCAM</td>
<td></td>
</tr>
<tr>
<td>PMS2</td>
<td>4</td>
</tr>
</tbody>
</table>

High-risk breast/ovarian

<table>
<thead>
<tr>
<th>Gene</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td></td>
</tr>
<tr>
<td>BMPR1A</td>
<td></td>
</tr>
<tr>
<td>SMAD4</td>
<td></td>
</tr>
<tr>
<td>CDK4</td>
<td></td>
</tr>
<tr>
<td>CDKN2A</td>
<td>1</td>
</tr>
<tr>
<td>PALLD</td>
<td></td>
</tr>
<tr>
<td>MET</td>
<td></td>
</tr>
<tr>
<td>MEN1</td>
<td></td>
</tr>
<tr>
<td>RET</td>
<td></td>
</tr>
<tr>
<td>PTCH1</td>
<td></td>
</tr>
<tr>
<td>VHL</td>
<td></td>
</tr>
<tr>
<td>MUTYH</td>
<td>23</td>
</tr>
</tbody>
</table>

Moderate-risk breast/ovarian

Lynch Syndrome

Other cancers
Clinical relevance

• For 80% of non-BRCA positives, the patient’s cancer and/or family history was consistent with the known effects of the gene they carried.
  – Even when the proband did not present with a canonical tumor type

• However, in many of these cases the patient would not have been tested for that gene under current guidelines.

• The other 20% of patients could have:
  – Rare pleiotropic effects
  – Incomplete family histories
  – Cancers unrelated to their genetics (with the possibility of a linked cancer in their future)
<table>
<thead>
<tr>
<th>Intervention Warranted based on gene and/or risk level</th>
<th>Recommend MRI(^c) (&gt;20% risk of breast cancer(^d))</th>
<th>Recommend RRSO</th>
<th>Discuss Option of RRM</th>
</tr>
</thead>
<tbody>
<tr>
<td>ATM, BRCA1, BRCA2, CDH1, CHEK2, PALB2, PTEN, STK11, TP53</td>
<td>BRCA1, BRCA2, Lynch syndrome(^e)</td>
<td>BRCA1, BRCA2, CDH1, PTEN, TP53</td>
<td></td>
</tr>
</tbody>
</table>

| Insufficient evidence for intervention\(^b\) | BARD1, BRIP1 | BARD1, BRIP1, PALB2, RAD51C, RAD51D | ATM, BARD1, CHEK2, PALB2, STK11 |

\(^b\) Intervention may still be warranted based on family history or other clinical factors.

\(^a\) Other genes may be included in multi-gene testing.

\(^b\) Intervention may still be warranted based on family history or other clinical factors.

\(^c\) See NCCN Guidelines for Breast Cancer Screening and Diagnosis.

\(^d\) May be modified based on family history or specific gene mutation.

\(^e\) See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal.
Preliminary observations for non-BRCA findings

Clinical actionability

- **55%** of the non-BRCA findings would warrant consideration of a *change in care for the patient.*
  - Under current medical guidelines
  - Over and above actions, based on family history alone
  - Specific recommendations varied considerably.

- **70%** of the non-BRCA findings would warrant consideration of a *change in care for positive family members.*
  - Thus, family member testing is indicated.

For details: Ellisen, *et al.* ASCO Meeting. 2015 (June).
Conclusions

1. Next-generation sequencing can equal performance of traditional methods.
   - But not “out of the box”
   - Takes a lot of work

2. It is possible to produce similar interpretations.
   - As some big established labs with proprietary data
   - In most cases

3. Expanded panels tests have clinical relevance and clinical utility.

All data have been deposited to the ClinVar database.
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