Multigene Panel Testing for Hereditary Cancer Risk

Dana Zakalik, M.D.
Director, Nancy and James Grosfeld Cancer Genetics Center
Professor, OUWB Medical School
MCC Annual Meeting
November 4, 2015
Outline

• Background
• New technologies
• “New” genes
• Panel testing 2015
• Management implications (?)
• Case examples
• ASCO Policy Statement
• Future horizons
Background

- 1980’s and 1990’s discovery of rare, highly penetrant cancer predisposition genes
  - *BRCA1* and 2, *p53*, *APC*
- 100+ known cancer susceptibility syndromes
- Significant recent changes in practice
- Next-generation sequencing (NGS) technology
- Multiplex gene-panel testing
Definition of Terms

• Next-generation sequencing (NGS)
  – Massively parallel sequencing
  – High throughput parallel sequencing of thousands to millions of fragments of DNA

• Multiplex gene-panel testing
  – Targeted analysis of multiple genes of interest simultaneously using NGS

• Variant of unknown significance (VUS)
  – Genetic sequence change whose association with disease is currently unknown
Random fragmentation

Ligation adapters

Size selection of fragments

Amplified solid state

Goal: increasing the signal of each unique molecule by clonal amplification (can be done different ways)

- Beads with complementary adapters
- Emulsion PCR: one template per bead per cell droplet = clonal amplification
- Clonal amplification of fixed template into clusters
- Bridge amplification

Goal: measure the addition of complementary bases (different chemistries used)

- pH read-out
- Light read-out
Gene Panel Testing

- Allows for efficient analysis of multiple genes
- Next generation sequencing (NGS) technology
  - Rapid, simultaneous gene analysis
- Available for multiple genetic syndromes
- Caveat: Variants of uncertain significance (VUS)
  - Potential for misinterpretation
  - May lead to confusion re: management of risk
- Clinical utility not proven – which genes are “actionable”?
- Potential for uncertainty re: optimal management
- Lack of evidence-based guidelines for many genes
- Genetic evaluation/counseling imperative
Multiplex Genetic Testing for Cancer Susceptibility:
Out on the High Wire Without a Net?

Susan M. Domchek and Angela Bradbury, University of Pennsylvania, Philadelphia, PA
Judy E. Garber, Dana-Farber Cancer Institute, Boston, MA
Kenneth Offit and Mark E. Robson, Memorial Sloan-Kettering Cancer Center and Weill Cornell Medical College, New York, NY
Table 1. Genes Analyzed in Commercially Available Multiplex Panels

<table>
<thead>
<tr>
<th>Gene</th>
<th>CancerNext</th>
<th>BreastNext</th>
<th>ColoNext</th>
<th>OvaNext</th>
<th>BROCA</th>
<th>ColoSeq</th>
</tr>
</thead>
<tbody>
<tr>
<td>APC</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ATR</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAP1M1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAP1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BARD1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMPR1A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BRF1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDH1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CEK4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDKN2A</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHEK1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CHEK2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAM175A/Aporaxas</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSH11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSH12</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MSH6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MUTYH</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NER</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PALB2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PMS2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PRSS1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAD50</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAD51</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAD51B</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAD51C</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RAD51D</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RBEB8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RET</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMAD4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STK11</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPS3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TPS3BP1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>UMC1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VHL</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XFC2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XFC3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Also Viejo, CA.
†Seattle, WA.
Gene Panel Testing

• Multiple labs offering a variety of different combinations of genes including: RAD 51D, RAD 51C, BRIP1, NBN, BARD1, CHEK2, PTEN, CDH1.....
  – Lack of management guidelines
• Increasingly utilized in clinical and academic setting
• 4-16% prevalence of non-\textit{BRCA1/2} mutations reported
  – 11% in Beaumont series
• Potential for clinical benefit (?)
• Uncertainty regarding gene panel selection, interpretation of results
  – High VUS rate
• Need further research, sharing of data ("PROMPT")
Two Decades After *BRCA*: Setting Paradigms in Personalized Cancer Care and Prevention

Fergus J. Couch, Katherine L. Nathanson, Kenneth Offit

28 March 2014 VOL 343 SCIENCE www.sciencemag.org
The ‘Other’ Breast Cancer Genes
The “Other” Ovarian Cancer Genes

![Pie chart showing Germline HR mutations with BRCA1 at 54%, BRCA2 at 21%, BARD1 at 2%, BRIP1 at 5%, CHEK1 at 1%, CHEK2 at 4%, FAM175A at 2%, NBN at 1%, PALB2 at 2%, RAD51C at 3%, and RAD51D at 5%]
# Beyond BRCA: Other Hereditary Breast Cancer Syndromes

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Key Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cowden Syndrome <em>(PTEN)</em></td>
<td>Breast, Uterine, Thyroid Cancers; Large head size; Skin findings</td>
</tr>
<tr>
<td>Li Fraumeni Syndrome <em>(LFS p53)</em></td>
<td>Breast, Brain, and Lung Cancers, Sarcomas, Adrenocortical Carcinoma; very early age at diagnosis</td>
</tr>
<tr>
<td><em>PALB2</em></td>
<td>Breast and Pancreatic Cancers (? Ovary)</td>
</tr>
<tr>
<td>Hereditary Diffuse Gastric Cancer <em>(HDGC CDH1)</em></td>
<td>Breast and Stomach Cancers</td>
</tr>
<tr>
<td>Peutz Jeghers Syndrome <em>(PJS)</em> <em>(STK11)</em></td>
<td>Breast, Colon, Pancreatic and Stomach Cancers; Freckling of lips in childhood</td>
</tr>
</tbody>
</table>
Multigene Panel Testing Detects Equal Rates of Pathogenic \textit{BRCA1/2} Mutations and has a Higher Diagnostic Yield Compared to Limited \textit{BRCA1/2} Analysis Alone in Patients at Risk for Hereditary Breast Cancer

Nimmi S. Kapoor, MD\textsuperscript{1,2}, Lisa D. Curcio, MD, FACS\textsuperscript{2}, Carlee A. Blakemore, BS\textsuperscript{1}, Amy K. Bremner, MD\textsuperscript{3}, Rachel E. McFarland, BS\textsuperscript{4}, John G. West, MD, FACS\textsuperscript{1}, and Kimberly C. Banks, MS, CGC, MBA\textsuperscript{4}

• 966 patients
  – 629 limited testing (BRCA1/2)
  – 337 panel testing (5-43 genes, 15 avg)

• Deleterious BRCA 1/2 mutations found in 4.0% (limited) and 3.6% (panel)

• Multigene panel testing identified additional 3.9% non-BRCA mutations

• Most common non-BRCA: PALB2, CHEK2, ATM
FIG. 1 Distribution of pathogenic mutations identified in panel group. a Separation by BRCA1/2 status. b Incidence of non-BRCA1/2 gene mutations
Management of Moderate Risk Genes

• Paucity of evidence-based guidelines
• Risk not fully defined
• Actionability: lack of consensus
• High rate of variants of unknown significance
• Implication for family members who test negative? May still be high risk
FIG. 2 Distribution of variants of uncertain significance in panel group

Frequency of Variants in Panel Group

<table>
<thead>
<tr>
<th>Gene</th>
<th>Number of Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>TP53</td>
<td></td>
</tr>
<tr>
<td>STK11</td>
<td></td>
</tr>
<tr>
<td>RAD51</td>
<td></td>
</tr>
<tr>
<td>RAD50</td>
<td></td>
</tr>
<tr>
<td>PTEN2</td>
<td></td>
</tr>
<tr>
<td>NF1</td>
<td></td>
</tr>
<tr>
<td>NBN</td>
<td></td>
</tr>
<tr>
<td>MUTYH</td>
<td></td>
</tr>
<tr>
<td>MRE11A</td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td></td>
</tr>
<tr>
<td>CHEK2</td>
<td></td>
</tr>
<tr>
<td>CDH1</td>
<td></td>
</tr>
<tr>
<td>BRIP1</td>
<td></td>
</tr>
<tr>
<td>BRCA2</td>
<td></td>
</tr>
<tr>
<td>BRCA1</td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td></td>
</tr>
<tr>
<td>APC</td>
<td></td>
</tr>
</tbody>
</table>

Number of Patients
Original Investigation

Clinical Actionability of Multigene Panel Testing for Hereditary Breast and Ovarian Cancer Risk Assessment

Andrea Desmond, BS; Allison W. Kurian, MD, MSc; Michele Gabree, MS, CGC; Meredith A. Mills, BA;
Michael J. Anderson, PhD; Yuya Kobayashi, PhD; Nora Horick, MS; Shan Yang, PhD; Kristen M. Shannon, MS, CGC;
Nadine Tung, MD; James M. Ford, MD; Stephen E. Lincoln, BS; Leif W. Ellisen, MD, PhD

**Importance** The practice of genetic testing for hereditary breast and/or ovarian cancer (HBOC) is rapidly evolving owing to the recent introduction of multigene panels. While these tests may identify 40% to 50% more individuals with hereditary cancer gene mutations than does testing for BRCA1/2 alone, whether finding such mutations will alter clinical management is unknown.

**Objective** To define the potential clinical effect of multigene panel testing for HBOC in a clinically representative cohort.

**Design, Setting, and Participants** Observational study of patients seen between 2001 and
Actionability of Panel Testing (JAMA Oncol 2015)

• 1046 pts seen for genetic testing (BRCA neg)
  – Stanford, MGH, BIDMC  2001-2014
• Multigene panel testing (25-29 genes)
• 40 pts (3.8%) harbored deleteriouserious mutations
• Most commonly: CHEK2, ATM, PALB2
• Additional screening/prevention offered
  – Management change considered in 33 pts
• Guidelines for management ??
Table 2. Management Change for Patients and Their Family Members Following Positive Multigene Panel Patient Findings

<table>
<thead>
<tr>
<th>Intervention Criteria</th>
<th>Relevant Genes$^a$</th>
<th>Intervention</th>
<th>Patients Recommended for Intervention, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-risk genes, NCCN management guidelines (n=20)</td>
<td>CDH1, TP53, PTEN, MLH1, MSH2, MSH6, PMS2, APC, MUTYH (biallelic), BMPR1A</td>
<td>Guidelines-based surveillance, prevention</td>
<td>20</td>
</tr>
<tr>
<td>Breast cancer risk ≥40% (&lt;40% pretest risk by IBIS$^c,d$) (n=8)</td>
<td>PALB2$^d$</td>
<td>Surgical prevention candidate</td>
<td>5$^e$</td>
</tr>
<tr>
<td>Breast cancer risk &gt;20%/NCCN I.2015 recommendations$^f$ (&lt;20% pre-test risk by IBIS$^c,d$) (n=32)</td>
<td>ATM,$^f$ CHEK2,$^f$ BRIP1, NBN, RAD51C</td>
<td>Enhanced breast screening candidate</td>
<td>5</td>
</tr>
<tr>
<td>Other cancer risk (pancreas, melanoma) (n=3)</td>
<td>CDKN2A</td>
<td>Pancreas screening candidate</td>
<td>3</td>
</tr>
</tbody>
</table>

Abbreviations: IBIS, Informed Biologic Interactions Score; NCCN, National Comprehensive Cancer Network.$^a$ Only genes with a guidelines-based surveillance, prevention, surgical prevention candidate, enhanced breast screening candidate, or pancreas screening candidate recommendation in the guidelines were included. $^b$ Risk estimates considered in the example.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Risk Category</th>
<th>Management Change Considered&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Considered Change</th>
<th>Family Testing Considered&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH1 (n=4)</td>
<td>High-risk BR/OV</td>
<td>4 of 4</td>
<td>Prophylactic gastrectomy</td>
<td>4 of 4</td>
</tr>
<tr>
<td>TP53 (n=3)</td>
<td>High-risk BR/OV</td>
<td>3 of 3</td>
<td>Increased cancer surveillance</td>
<td>3 of 3</td>
</tr>
<tr>
<td>PTEN (n=1)</td>
<td>High-risk BR/OV</td>
<td>1 of 1</td>
<td>Increased cancer surveillance</td>
<td>1 of 1</td>
</tr>
<tr>
<td>ATM (n=11)&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Mod-/low-risk BR/OV</td>
<td>1 of 11</td>
<td>Increased breast screening</td>
<td>6 of 11</td>
</tr>
<tr>
<td>BRIP1 (n=1)</td>
<td>Mod-/low-risk BR/OV</td>
<td>0 of 1</td>
<td>NA</td>
<td>0 of 1</td>
</tr>
<tr>
<td>CHEK2 (n=15)</td>
<td>Mod-/low-risk BR/OV</td>
<td>2 of 15</td>
<td>Increased breast screening</td>
<td>4 of 13</td>
</tr>
<tr>
<td>NBN (n=2)</td>
<td>Mod-/low-risk BR/OV</td>
<td>0 of 2</td>
<td>NA</td>
<td>0 of 1</td>
</tr>
<tr>
<td>PALB2 (n=8)</td>
<td>Mod-/low-risk BR/OV</td>
<td>5 of 8</td>
<td>Increased screening or mastectomy</td>
<td>7 of 7</td>
</tr>
<tr>
<td>RAD51C (n=3)</td>
<td>Mod-/low-risk BR/OV</td>
<td>2 of 3</td>
<td>Increased breast screening</td>
<td>3 of 3</td>
</tr>
<tr>
<td>MLH1 (n=1)</td>
<td>Lynch syndrome</td>
<td>1 of 1</td>
<td>Increased colorectal/endometrial screening</td>
<td>1 of 1</td>
</tr>
<tr>
<td>MSH2 (n=2)</td>
<td>Lynch syndrome</td>
<td>2 of 2</td>
<td>Increased colorectal/endometrial screening</td>
<td>1 of 1</td>
</tr>
<tr>
<td>MSH6 (n=2)</td>
<td>Lynch syndrome</td>
<td>2 of 2</td>
<td>Increased colorectal/endometrial screening</td>
<td>2 of 2</td>
</tr>
<tr>
<td>PMS2 (n=4)</td>
<td>Lynch syndrome</td>
<td>4 of 4</td>
<td>Increased colorectal screening</td>
<td>4 of 4</td>
</tr>
<tr>
<td>APC (n=1)</td>
<td>Other familial cancer</td>
<td>1 of 1</td>
<td>Prophylactic colectomy</td>
<td>1 of 1</td>
</tr>
<tr>
<td>BMPR1A (n=1)</td>
<td>Other familial cancer</td>
<td>1 of 1</td>
<td>Increased gastric cancer screening</td>
<td>1 of 1</td>
</tr>
<tr>
<td>CDKN2A (n=3)</td>
<td>Other familial cancer</td>
<td>3 of 3</td>
<td>Increased pancreatic surveillance</td>
<td>3 of 3</td>
</tr>
<tr>
<td>MUTYH (n=1)</td>
<td>Other familial cancer</td>
<td>1 of 1</td>
<td>Increased colorectal screening</td>
<td>1 of 1</td>
</tr>
<tr>
<td>Total (n=63)</td>
<td>NA</td>
<td>33 of 63</td>
<td>NA</td>
<td>42 of 58</td>
</tr>
<tr>
<td>Intervention Warranted based on gene and/or risk level</td>
<td>Recommend MRI&lt;sup&gt;6&lt;/sup&gt; (&gt;20% risk of breast cancer&lt;sup&gt;d&lt;/sup&gt;)</td>
<td>Recommend RRSO</td>
<td>Discuss Option of RRM</td>
<td></td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>---------------------------------------------------------------</td>
<td>----------------</td>
<td>-------------------</td>
<td></td>
</tr>
<tr>
<td>ATM BRCA1 BRCA2 CDH1 CHEK2 PALB2 PTEN STK11 TP53</td>
<td>BRCA1 BRCA2 Lynch syndrome&lt;sup&gt;e&lt;/sup&gt;</td>
<td>BRCA1 BRCA2</td>
<td>CDH1 PTEN TP53</td>
<td></td>
</tr>
<tr>
<td>Insufficient evidence for intervention&lt;sup&gt;b&lt;/sup&gt;</td>
<td>BARD1 BRIP1</td>
<td>BARD1 BRIP1</td>
<td>BARD1 CHEK2 PALB2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>PALB2 RAD51C</td>
<td>RAD51D STK11</td>
<td></td>
</tr>
</tbody>
</table>
Usefulness of Multigene Testing
Catching the Train That’s Left the Station

Elizabeth M. Swisher, MD

In the mid-1990s, clinical testing for BRCA1 and BRCA2 mutations rapidly followed gene cloning. Given the lack of clinical experience with BRCA1 and BRCA2 testing, many cancer genetics experts spoke against clinical testing, advocating that testing be done in the research setting only. Increasingly, women and their physicians ignored those recommendations, and testing expanded beyond very high-risk patients. In line with previous studies, the authors identified 3.8% of BRCA1/2-negative cases to have deleterious mutations in other cancer-risk genes. Reassuringly, more than 90% of these mutations occurred in genes consistent with the personal or family history, demonstrating the clinical relevance of these results and the relative rarity of incidental mutations. Importantly, the majority of mutations resulted in changes in recommendation for cancer screening or prevention. It is important to realize that participants in this study had predominantly...
Clinical application of multigene panels: challenges of next-generation counseling and cancer risk management

Edited by:
Pamela Pollock,
Institute for Health and Biomedical Innovation, Australia

Thomas Paul Slavin*, Mariana Niell-Swiller, Ilana Solomon, Bita Nehoray, Christina Rybak, Kathleen R. Blazer and Jeffrey N. Weitzel*

Division of Clinical Cancer Genetics, Department of Medical Oncology, City of Hope, Duarte, CA, USA
These slides are the property of the presenter. Do not duplicate without express written consent.
Overview of multi-gene testing

- The recent introduction of multi-gene testing for hereditary forms of cancer has rapidly altered the clinical approach to testing at-risk patients and their families. Based on next-generation sequencing technology, these tests simultaneously analyze a set of genes that are associated with a specific family cancer phenotype or multiple phenotypes.
- Patients who have a personal or family history suggestive of a single inherited cancer syndrome are most appropriately managed by genetic testing for that specific syndrome. When more than one gene can explain an inherited cancer syndrome, than multi-gene testing, may be more efficient and/or cost-effective.
- There is also a role for multi-gene testing in individuals who have tested negative (indeterminate) for a single syndrome, but whose personal or family history remains strongly suggestive of an inherited susceptibility.
- As commercially available tests differ in the specific genes analyzed (as well as classification of variants and many other factors), choosing the specific laboratory and test panel is important.
- Multi-gene testing can include “intermediate” penetrant (moderate-risk) genes. For many of these genes, there are limited data on the degree of cancer risk and there are no clear guidelines on risk management for carriers of mutations. Not all genes included on available multi-gene tests are necessarily clinically actionable. As is the case with high-risk genes, it is possible that the risks associated with moderate-risk genes may not be entirely due to that gene alone, but may be influenced by gene/gene or gene/environment interactions. Therefore, it may be difficult to use a known mutation alone to assign risk for relatives. In many cases the information from testing for moderate penetrance genes does not change risk management compared to that based on family history alone.
- There is an increased likelihood of finding variants of unknown significance when testing for mutations in multiple genes.
- It is for these and other reasons that multigene testing are ideally offered in the context of professional genetic expertise for pre- and post-test counseling.
<table>
<thead>
<tr>
<th>Intervention Warranted based on gene and/or risk level</th>
<th>Recommend MRI ((\gt 20%) risk of breast cancer)</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(^a)</td>
<td>BRCA1, BRCA2, CDH1, PTEN, TP53</td>
<td></td>
</tr>
<tr>
<td>Insufficient evidence for intervention(^b)</td>
<td>BARD1, BRIP1</td>
<td>BARD1, BRIP1, PALB2, RAD51C, RAD51D</td>
<td>ATM, BARD1, CHEK2, PALB2, STK11</td>
</tr>
</tbody>
</table>
EXAMPLES OF ADDITIONAL GENETIC MUTATIONS ASSOCIATED WITH BREAST/OVARIAN CANCER RISK

• Hereditary Diffuse Gastric Cancer Syndrome ([See NCCN Guidelines for Gastric Cancer](#))
  › CDH1 gene
  › Diffuse gastric cancer — 67%–83% risk
  › Lobular cancer of the breast — 39%–52% risk

• Peutz-Jeghers Syndrome ([See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal](#) for more information)
  › STK11/LKB1 gene
  › Breast cancer — 44%–50% risk
  › Ovarian cancer — 18%–21% risk (ovarian sex cord tumors are the most common)

• Lynch Syndrome ([See NCCN Guidelines for Genetic/Familial High-Risk Assessment: Colorectal](#) for more information)
  › Mismatch Repair (MMR) genes — MLH1, MSH2, MSH6, PMS2
  › EPCAM gene deletion
  › Ovarian cancer — 9% risk
  › Breast cancer — conflicting data regarding increased risks
Positive result. Pathogenic variant identified in MRE11A.

Clinical Summary

- A pathogenic variant, c.1441delA (p.Thr481Hisfs*43), was identified in MRE11A.
- The MRE11A gene is associated with an increased risk for autosomal dominant breast cancer in individuals who carry a single pathogenic variant (PMID: 14684699, 24894818). To date, the data is limited and cancer risk is not clearly established. MRE11A is also associated with autosomal recessive ataxia-telangiectasia-like disorder (ATLD) (MedGen UID: 348929).
- Variants in MRE11A have been associated with an increased susceptibility to breast cancer. The data and sample sizes, however, are limited and are based on small studies. Consequently, lifetime risk estimates are not well established (PMID: 14684699, 24894818). Elevated risk for gynecologic cancers has been considered but available evidence is not sufficient to make a determination at this time.
- Close relatives (children, siblings, and each parent) have up to a 50% chance of being a carrier of this pathogenic variant. More distant relatives may also be carriers. Carriers are at increased risk of developing MRE11A-related cancers and may have reproductive risks related to autosomal recessive MRE11A-related ATLD as well. Testing for this variant is available.
- These results should be interpreted within the context of additional laboratory results, family history, and clinical findings. Genetic counseling is recommended to discuss the implications of this result. For a listing of genetic counselors, please visit www.nsgc.org.

Complete Results

<table>
<thead>
<tr>
<th>Gene</th>
<th>Condition Group</th>
<th>Variant</th>
<th>Zygosity</th>
<th>Variant Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRE11A</td>
<td>Hereditary Cancers (Breast and Ovarian)</td>
<td>c.1441delA (p.Thr481Hisfs*43)</td>
<td>heterozygous</td>
<td>PATHOGENIC</td>
</tr>
</tbody>
</table>

The following genes were evaluated for sequence changes and exonic deletions/duplications: ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, FANCC, MRE11A, NBN, NFI, PALB2, PTEN, RAD50, RAD51C, STK11, TP53

Results are negative unless otherwise indicated.

Benign variants, and silent and intronic variants with no evidence towards pathogenicity, are not included in this report but are available upon request.
**OvaNext:** Analyses of 23 Genes Associated with Hereditary Ovarian Cancer

**PANEL RESULTS**

**PALB2**

Pathogenic Mutation(s):  p.Q66*

**SUMMARY**

**POSITIVE: Pathogenic Mutation Detected**

**INTERPRETATION**

- This individual is heterozygous for the p.Q66* pathogenic mutation in the PALB2 gene.

- **Cancer Risk estimate:** 33-58% lifetime risk for breast cancer (females only) and increased lifetime pancreatic cancer risk++

- The expression and severity for this individual cannot be predicted.

- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected in the other genes analyzed. In total, 23 genes were analyzed as part of this panel: ATM, BARD1, BRCA1, BRCA2, BRIp1, CDH1, CHEK2, EPCAM, MLH1, MRE11A, MSH2, MSH6, MUTYH, NBN, NF1, PALB2, PMS2, PTEN, RAD50, RAD51C, RAD51D, STK11, and TP53.

Breast-Cancer Risk in Families with Mutations in **PALB2**


**The New York Times**

**HEALTH**

**Study Shows Third Gene as Indicator for Breast Cancer**

By NICHOLAS BAKALAR  AUG. 6, 2014

These slides are the property of the presenter. Do not duplicate without express written consent.
**PALB2 and Cancer Risk**

- **Breast Cancer Risk** (association with triple negative type)
  - 14% by age 50
  - 35% by age 70
  - Impact of family history
    - 33%-58%

- **Pancreas Cancer Risk**
  - Identified in 3-4% of familial pancreatic cancer cases

- **Ovarian Cancer Risk** – conflicting results

- **Other cancers (?)**
Specific Site Analysis of BRIP1

RESULTS

BRIP1 SPECIFIC SITE  c.2038_2039dupTT Pathogenic Mutation: Not Detected

INTERPRETATION

This individual does not carry the **c.2038_2039dupTT** pathogenic mutation in the **BRIP1** gene, which was previously identified in this individual’s relative(s). Therefore, this individual is not expected to be at an increased risk for **BRIP1**-related cancers due to this familial mutation.
Summary

Positive result: **Homozygous** Pathogenic variant identified in CDKN2A.

Clinical Summary

- A homozygous, Pathogenic variant, c.148C>T (p.Gln50*), was identified in CDKN2A.
  - The CDKN2A gene is associated with autosomal dominant cutaneous melanoma (MedGen UID: 268851).
  - This result is consistent with a predisposition to, or diagnosis of, CDKN2A-related conditions.
  - Hereditary cutaneous melanoma is characterized by multiple melanocytic nevi and is a hereditary cancer syndrome that increases an individual’s lifetime risk of developing cutaneous melanoma. CDKN2A sequence changes have also been associated with increased risk for pancreatic cancers, and some studies have reported increased incidence of breast cancer (PMID: 10922411, 15879498, 16905682, 17047042, 18981015, 22636603). Individuals who are homozygous for Pathogenic variants in CDKN2A are uncommon, but have been reported in the literature (PMID: 7670475).
  - It is likely that one copy of this Pathogenic variant was inherited from each parent. All children of this individual have a 100% chance to inherit a Pathogenic variant. More distant relatives may also be carriers. Testing for this variant is available.

- In addition, a Variant of Uncertain Significance, c.1008C>A (p.His336Gln), was also identified in PALLD.
  - The PALLD gene has limited evidence supporting a direct causal role in the susceptibility to pancreatic cancer (MedGen UID: 339739, OMIM: 606856). Consequently, the majority of PALLD variants identified to date are classified as Uncertain Significance.
  - The clinical significance of this variant is uncertain at this time. Until this uncertainty can be resolved, caution should be exercised before using this result to inform clinical management decisions.
  - Based on our current review of the medical literature there is only limited evidence available to support this gene having a direct causal role for pancreatic cancer. Therefore variants in this gene are not eligible for family tracking as a part of our VUS Resolution Program at this time. Please visit www.invitae.com for more information on our VUS Resolution Program.

- These results should be interpreted within the context of additional laboratory results, family history, and clinical findings. Genetic counseling is recommended to discuss the implications of this result. For a listing of genetic counselors, please visit www.geneticcounselors.org or contact a professional organization.
**BreastNext: Analyses of 18 Genes Associated with Hereditary Breast Cancer**

**TP53**

**Pathogenic Mutation(s):** p.R337H

**SUMMARY**

**POSITIVE: Pathogenic Mutation Detected**

**INTERPRETATION**

- This individual is heterozygous for the p.R337H pathogenic mutation in the TP53 gene.
- This result is consistent with a diagnosis of Li-Fraumeni syndrome (LFS).
- **Cancer Risk estimate:** lifetime cancer risk of up to 93%++
- The expression and severity for this individual cannot be predicted.
- Genetic counseling is a recommended option for all individuals undergoing genetic testing.

No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected in the other genes analyzed. In total, 18 genes were analyzed as part of this panel: **ATM, BARD1, BRCA1, BRCA2, BRIP1, CDH1, CHEK2, MRE11A, MUTYH, NBN, NF1, PALB2, PTEN, RAD50, RAD51C, RAD51D, STK11, and TP53.**

The p.R337H pathogenic mutation (also known as c.1010G>A) is located in coding exon 9 of the TP53 gene. This alteration results from a G to A substitution at nucleotide position 1010. The arginine at codon 337 is replaced by histidine, an amino acid with highly similar properties. There currently exists a relatively extensive body of literature regarding this alteration. It has been detected at high frequency in Brazilian LFS families and is highly associated with a common haplotype, providing strong evidence of a founder effect in this population (Garrermo et al. *Hum Mutat.* 2010 Feb;31(2):143-50). Ribeiro and colleagues described p.R337H as low-penetrance mutation, primarily associated with adrenal cortical tumor risk in childhood (Proc Natl Acad Sci U S A. 2001 Jul 31;98(16):9330-5). However, other studies have led authors to conclude that the lifetime cancer risks for carriers of this mutation are similar to those of other LFS families, although p.R337H-associated cancers tend to occur with a later age of onset (Garrermo et al. *Hum Mutat.* 2010 Feb;31(2):143-50). Families with this mutation can present with a wide spectrum of tumors including, but not limited to, breast cancers, brain cancers, soft tissue sarcomas, and adrenocortical carcinoma (Achatz et al. *Cancer Lett.* 2007 Jan 8;246(1-2):96-102; IARC Database http://www-p53.iarc.fr/). This amino acid substitution occurs within the protein tetramerization domain and alters the ability of TP53 to form stabilizing oligomers with high DNA-binding capacity in cells with elevated pH levels. This pH dependence may explain the incomplete penetrance observed in many families carrying the p.R337H mutation and has led to the hypothesis that p.R337H is a conditional mutant (DiGiampietro et al. *NAT STRUCBIOI.* 2002, 9:12-6; Giacomazzi J, et al. *BMC Cancer* 2013 Apr;13(1):187). Given the inter-familial variability in penetrance and tumor patterns described to date, some have suggested that surveillance recommendations for p.R337H carriers should be based on family cancer history (Palermo et al. *Cancer Lett.* 2008 Mar 8;261(1):21-5). Based on the available evidence, p.R337H is classified as a pathogenic mutation.

**These slides are the property of the presenter. Do not duplicate without express written consent.**
**BRCAplus: Analyses of 5 High-Risk Hereditary Breast Cancer Genes**

**PANEL RESULTS**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Pathogenic Mutation</th>
</tr>
</thead>
<tbody>
<tr>
<td>CDH1</td>
<td>c.1979dupT</td>
</tr>
</tbody>
</table>

**SUMMARY**

**POSITIVE: Pathogenic Mutation Detected**

**INTERPRETATION**

- This individual is heterozygous for the c.1979dupT pathogenic mutation in the CDH1 gene.
- This result is consistent with a diagnosis of hereditary diffuse gastric cancer (HDGC).
- **Cancer Risk estimate:** lifetime risks of 67-83% for diffuse gastric cancer and 39-52% for lobular breast cancer (females only)**+
- The expression and severity for this individual cannot be predicted.
- Genetic counseling is a recommended option for all patients undergoing genetic testing.

No pathogenic mutations, variants of unknown significance, or gross deletions or duplications were detected in the other genes analyzed. In total, 5 genes were analyzed as part of this panel: BRCA1, BRCA2, CDH1, PTEN, and TP53.

The c.1979dupT pathogenic mutation, located in coding exon 13 of the CDH1 gene, results from a duplication of T at position 1979, causing a translational frameshift with a predicted alternate stop codon. Since frameshifts are typically deleterious in nature, this alteration is interpreted as a disease-causing mutation (ACMG Recommendations for Standards for Interpretation and Reporting of Sequence Variations. Revision 2007. Genet Med. 2008;10:294).

*These slides are the property of the presenter. Do not duplicate without express written consent.*
SGO Clinical Practice Statement: Next Generation Cancer Gene Panels Versus Gene by Gene Testing

March 2014

The advent of next generation sequencing has led to an era of inexpensive, high throughput DNA sequencing, which is having a major impact on both cancer research and clinical care. Cancer gene panels use next generation sequencing technology to assess inherited mutations in multiple genes simultaneously and are currently commercially available. The unanimous decision by the U.S. Supreme Court in June, 2013 to invalidate human gene patents led to a rapid increase in vendors and expansion of clinical options for genetic testing. Current cancer gene panels vary in size from just two genes (i.e., BRCA1 and BRCA2) to larger panels that include more than 50 genes.

Until recently, most testing for cancer genetic risk involved traditional exon-by-exon Sanger sequencing of each candidate gene. In contrast, next generation sequencing allows parallel sequencing of millions of short pieces of DNA simultaneously. Prior to next generation sequencing, genetic testing usually started with the most commonly involved genes and proceeded to less likely genes only when clinical suspicion was very high. However, cancer panels allow testing of all genes in parallel without substantially increasing the cost, leading to a different clinical algorithm in which all known contributing genes can be assayed at first evaluation.
American Society of Clinical Oncology Policy Statement Update: Genetic and Genomic Testing for Cancer Susceptibility

Mark E. Robson, Angela R. Bradbury, Banu Arun, Susan M. Domchek, James M. Ford, Heather L. Hampel, Stephen M. Lipkin, Sapna Syngal, Dana S. Wollins, and Noralane M. Lindor
ASCO Recommendations 2015

• Germ-line implications of somatic profiling
• Multigene panel testing for hereditary cancer
• Quality assurance in genetic testing
• Education of Oncology professionals
• Access to cancer genetic services
Multigene Panel Testing ASCO

- Multigene (panel) testing is rapidly expanding
- Clinical utility of testing for moderate risk genes is not established
- Genetic counseling is important component
- ASCO encourages research to delineate optimal use of panel testing and development of evidence-based practice guidelines, and education of providers
ASCO Leadership Statement

• “Robust discussions among a diverse set of stakeholders will be needed to ensure that all perspectives are listened to and that genetic cancer susceptibility services are comprehensive and patient-centric.”

• Julie M. Vose, MD, MBA (President)
• Peter Yu, MD (Past president)
• Daniel F. Hayes, MD (President-Elect)