Germline testing for hereditary Cancer Syndromes
Update 2015
Topics to be discussed

- Case Presentation
- Standard Indications for testing common syndromes
- Next Generation Sequencing and panel testing
- Unique Challenges to genetic testing
- Conclusions for clinical management today
Family with Gastric Cancer and CDH1 mutation
CDH positive

- Rare
- 80% risk of gastric cancer by age 80
- Gastric cancer is submucosal and not detected on endoscopy
- Young lobular breast cancer
- Autosomal dominant
- Now a common “incidental“ finding in genetic panel testing
Hereditary Susceptibility to Cancer

- Who to test
- What test to select, and when to update testing on previously tested families
- What are the cancer risks associated with the mutation and how accurate are the estimates
- What interventions are indicated
- Is there evidence supporting improved clinical outcomes
When Should Genetic Testing Be Considered?

- Patient has a reasonable likelihood of carrying an altered cancer susceptibility gene
- Genetic test is available that can be adequately interpreted
- An affected individual is available for testing
- Results will influence medical management
- Patient wants information (empowerment)
<table>
<thead>
<tr>
<th>SYNDROME</th>
<th>Risk cancer</th>
<th>Recommendations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HBOC:</strong> Hereditary Breast and Ovarian Cancer</td>
<td>Breast 85%</td>
<td>MRI and Mammogram age 25 Prophylactic oophorectomy</td>
</tr>
<tr>
<td>BRCA 1 BRCA2</td>
<td>Ovary 40%</td>
<td></td>
</tr>
<tr>
<td><strong>Hereditary Colon Uterine</strong> LYNCH</td>
<td>Colon 80%</td>
<td>Colonoscopy yearly age 25 Gyn prophylactic surgery</td>
</tr>
<tr>
<td></td>
<td>Uterine 40%</td>
<td></td>
</tr>
<tr>
<td><strong>Hereditary Pancreatic Cancer</strong> PALB2, BRCA2</td>
<td>60% Pancreas breast 40%</td>
<td>Research screening</td>
</tr>
<tr>
<td><strong>Hereditary Gastric Cancer</strong> CDH</td>
<td>Gastric cancer 60-80%</td>
<td>Consider prophylactic surgery</td>
</tr>
</tbody>
</table>
Genetic testing in colorectal endometrial cancer families

As common as BRCA
Result can save lives with as much or more impact than BRCA
Testing today in a fraction of the candidate patients
Risk of Colorectal Cancer

- General population: 6%
- Personal history of colorectal neoplasia: 15%–20%
- Inflammatory bowel disease: 15%–40%
- HNPCC mutation: 70%–80%
- FAP/MAP: >95%

Lifetime risk (%)
DNA Mismatch Repair

Base pair mismatch

Normal DNA repair

Mutation introduced by unrepaired DNA
Testing Tumor for Lynch Syndrome

Tumor analysis - Screening for Lynch syndrome in the tumor (reliable in colon and uterine)
MSI - Microsatellite Instability
IHC - Immunohistochemistry

• Germline genetic testing

*MLH1, MSH2, MSH6, PMS2, EPCAMM*
Age-Related Penetrance

Percentage of individuals with altered mismatch repair gene who develop cancer.

- HNPCC mutation carriers
- General population

Affected with colorectal cancer (%)
Surveillance Improves HNPCC Survival

65% reduction in mortality

p = 0.05

Hereditary Breast and Ovarian Cancer

• Breast cancer before age 50
• Ovarian cancer at any age
• Male breast cancer at any age
• Multiple primary cancers
• Ashkenazi Jewish ancestry
• Relatives of a BRCA mutation carrier
• Triple Negative under age 60
Cancer Risks in Carriers of Germline Mutations in BRCA1 and BRCA2

Presented By Judy Garber at 2015 ASCO Annual Meeting
# Management Guidelines BRCA1/2 Carriers

<table>
<thead>
<tr>
<th>Management Option</th>
<th>Screening Interval/Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SCREENING</strong></td>
<td></td>
</tr>
<tr>
<td>• Clinical Breast Exam</td>
<td>• Q6-12 mos beginning age 25</td>
</tr>
<tr>
<td>• Breast MRI</td>
<td>• Yearly age 25-75 (then individualize)</td>
</tr>
<tr>
<td>• Mammogram</td>
<td>• Yearly age 30-75 (then individualize)</td>
</tr>
<tr>
<td>• Transvaginal ultrasound*</td>
<td>• Q6 mos beginning age 30</td>
</tr>
<tr>
<td>• CA-125*</td>
<td>• Q6 mos beginning age 30</td>
</tr>
<tr>
<td><strong>PREVENTION</strong></td>
<td></td>
</tr>
<tr>
<td>• Bilateral mastectomy</td>
<td>• Discuss option with patient</td>
</tr>
<tr>
<td>• Bilateral salpingo-oophorectomy</td>
<td>• Recommend by age 35-40 and when childbearing complete</td>
</tr>
<tr>
<td>• Consider oral contraceptive</td>
<td></td>
</tr>
<tr>
<td>• Consider tamoxifen</td>
<td></td>
</tr>
</tbody>
</table>
Updates in germline testing for breast and ovarian cancer families

- Large deletion and duplication testing
- Palb2
- Risk modifiers in Gene carriers
- Panel testing*
Large Duplication and Deletion analysis

- **BART**
  - Not done on most patient prior to 2012:
  - Routine sequencing: detecting single base changes, by Sanger sequencing
  - Rearrangement test: detects all large deletions and duplications of BRCA 1 and BRCA 2 coding: available since 2006, not routing

- **LRP**: 5-site large rearrangement panel in BRCA 1 available since 2002, routinely included
  - Identifies additional 2.3% overall, accounts for upto 9% of all mutations in high risk families
Core Genes in the Homologous Recombination-Repair Pathway

- SSB1
- MRE11
- NBN
- RAD50
- RINT1
- ATM
- CHEK2
- TP53
- BARD1
- BRCA1
- BRIP1
- PALB2
- BRCA2
- RAD51 Paralog complexes

Cell cycle arrest
- G2M
- S
- G1

Apoptosis
PALB-2

- Partner and Localizer of BRCA 2
- Homologous DNA repair
- Risk of Breast Cancer overall is 33% by age 70
- Risk is increased to 58% if family history greater than two first degree relatives
- accounts for 2.4% of familial aggregates of breast cancer
- Associated with increased risk of pancreatic cancer
# Breast Cancer Risk Factors

<table>
<thead>
<tr>
<th>Risk Factor</th>
<th>Relative Risk (RR)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparity/Late Parity</td>
<td>1.3</td>
</tr>
<tr>
<td>Early Menarch/Late Menopause</td>
<td>1.3</td>
</tr>
<tr>
<td>Alcohol Abuse</td>
<td>1.3</td>
</tr>
<tr>
<td>Hormone Replacement Therapy</td>
<td>1.4</td>
</tr>
<tr>
<td>Obesity</td>
<td>1.5</td>
</tr>
<tr>
<td>Female Age 65</td>
<td>1.7</td>
</tr>
<tr>
<td>Family History</td>
<td>1.7</td>
</tr>
<tr>
<td>Dense Breast Tissue</td>
<td>4.3</td>
</tr>
<tr>
<td>Biopsy with Atypia or LCIS</td>
<td>5.0</td>
</tr>
</tbody>
</table>
Breast Cancer and Ovarian Cancer and Panel Testing

- Testing has become clinically available on a widespread basis for only about 1-2 years
- Most labs offer wide variety of panels
- First data are recently published on results of panel testing
- Issues of VUS and estimates of cancer and incidental findings are common to all cancers
- Which panels, and what are the main issues
- What are the results in Breast, ovarian cancer in early use of testing
<table>
<thead>
<tr>
<th></th>
<th>Single Gene</th>
<th>Gene Panel</th>
<th>Exome</th>
<th>Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Target Size</strong></td>
<td>5000</td>
<td>500,000</td>
<td>50,000,000</td>
<td>3,000,000,000</td>
</tr>
<tr>
<td><strong>Method</strong></td>
<td>Sanger</td>
<td>Next Gen Seq</td>
<td>Next Gen Seq</td>
<td>Next Gen Seq</td>
</tr>
<tr>
<td></td>
<td>sequence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Gene Targets</strong></td>
<td>Selected</td>
<td>Selected</td>
<td>No selection bias</td>
<td>No Selection Bias</td>
</tr>
<tr>
<td><strong>Incidental Findings</strong></td>
<td>No</td>
<td>No</td>
<td>yes</td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Estimated Variants</strong></td>
<td>0-3</td>
<td>50</td>
<td>100,000</td>
<td>38,000,000</td>
</tr>
</tbody>
</table>
# NEJM 2015 Panel sequencing Breast Cancer Risk

<table>
<thead>
<tr>
<th>Gene</th>
<th>RR</th>
<th>Absolute risk</th>
<th>Breast and other</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>BRCA1</td>
<td>11.4</td>
<td>75%</td>
<td>Breasts ovary</td>
<td>BOADICA model</td>
</tr>
<tr>
<td>BRCA2</td>
<td>11</td>
<td>76%</td>
<td>Breast ovary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>prostate</td>
<td>prostate pancreas</td>
</tr>
<tr>
<td>TP53</td>
<td>105</td>
<td>Est 40%</td>
<td>Multiple cancers:</td>
<td>Li Fraumeni</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>sarcoma, adrenal</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>brain</td>
<td></td>
</tr>
<tr>
<td>PTEN</td>
<td></td>
<td>unknown</td>
<td>Thyroid, uterine,</td>
<td>Cowden’s syndrome very rare</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>clinical phenotype</td>
<td></td>
</tr>
<tr>
<td>CDH1</td>
<td>6.6</td>
<td>53%</td>
<td>Gastric lobular breast</td>
<td>Diffuse gastric syndrome</td>
</tr>
<tr>
<td>STK11</td>
<td></td>
<td></td>
<td>Stromal tumors</td>
<td>Peutz Jegher Very very rare</td>
</tr>
</tbody>
</table>
## NEJM 2015 Panel sequencing Breast Cancer Risk

<table>
<thead>
<tr>
<th>Gene</th>
<th>RR</th>
<th>Absolute Risk</th>
<th>Breast and other cancer</th>
<th>comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>PALB2</td>
<td>5.3</td>
<td>45%</td>
<td>Ovary unclear, pancreas</td>
<td></td>
</tr>
<tr>
<td>ATM</td>
<td>2.8</td>
<td>27%</td>
<td>Pancreas modest</td>
<td></td>
</tr>
<tr>
<td>CHEK2</td>
<td>2.7</td>
<td>29%</td>
<td>Possible</td>
<td>100Del c RR is only 1.3</td>
</tr>
<tr>
<td>NBN</td>
<td>2.7</td>
<td>23%</td>
<td>Biallelic associated leukemia</td>
<td>c.657del5 Slavic founder is data</td>
</tr>
<tr>
<td>MRE, RAD51</td>
<td>2.5</td>
<td>&gt;20%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Growing List of labs offering panel testing in genetics

- Ambry
- ApolloGen
- ARUP
- GeneDX
- Invitae
- ColorMe
- Myriad
- Pathway Genomics
- Prevention Genetics
- Quest
- University of Washington
- Fulgent Diagnostics
Is there concordance between labs?

• Validations not published by all labs
• Validation requirements not standardized
• Extent of analysis may differ (e.g. intronic depth)
• Argument in favor of increased regulatory oversight
Mutations

- Silent
- Missense: change in amino acid
- Truncating:
- Deletions, insertions = frameshift
- Splice site mutations alter donor or acceptor splice site causes incorrect splicing of exons
- Non sense: stop codon TAG, TAA TGA
# Types of Mutations

**Normal Message:**

THEBIGREDDOGRANOUT
THE BIG RED DOG RAN OUT

**Deletions:**

![Deletion Arrow]

THE BIR EDD OGR ANO UT

**Insertions:**

![Insertion Arrow]

THE BIG REB DOB GRA NOU TS
Variants of Unknown Significance

- THE BIG RED DOG RAN OUT
- THE BIG NED DOG RAN OUT
Making Sense of a VUS

1. Amino acid conservation (charge, polarity, volume, hydrophobicity, etc.) and Grantham Matrix Score (GMS)
2. Prevalence in a control population (SNP?)
3. Co-segregation with disease in affected families
4. Location within gene and protein functionality
5. In silico analysis (Align-GVGD, PolyPhen, SIFT, NNsplice)
6. Evolutionary conservation
7. Concurrent with known deleterious mutations
8. LOH of wild type allele in tumors
9. Locus-specific databases (BIC, LOVD, InSiGHT, MMR Genes Variant Database, IARC TP53 Database)
When should we test for a panel of cancer genes?

- This is a question that is not yet answered.
- When the results are actionable
- When the results can help families understand
- Mostly it is for future generations
- Only real standard test is in targeted gene
- Can be more cost effective if multiple genes are possible
- Consent for this information is complex
Panel Testing Results for Familial Breast Cancer

<table>
<thead>
<tr>
<th>Genes</th>
<th>N = # Patients with deleterious mutation (%)</th>
<th>95% Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Any deleterious mutation</td>
<td>11 (10.4)</td>
<td>5.30, 17.81</td>
</tr>
<tr>
<td>BRCA1 or BRCA2</td>
<td>7 (6.6)</td>
<td>2.70, 13.13</td>
</tr>
<tr>
<td>BRCA1</td>
<td>4 (3.8)</td>
<td>1.04, 9.38</td>
</tr>
<tr>
<td>BRCA2*</td>
<td>3 (2.8)</td>
<td>0.59, 8.05</td>
</tr>
<tr>
<td>Other genes related to breast cancer</td>
<td>5 (4.7)</td>
<td>1.55, 10.67</td>
</tr>
<tr>
<td>ATM*</td>
<td>2 (1.9)</td>
<td>0.23, 6.65</td>
</tr>
<tr>
<td>CHEK2</td>
<td>1 (0.9)</td>
<td>0.02, 5.14</td>
</tr>
<tr>
<td>PALB2</td>
<td>2 (1.9)</td>
<td>0.23, 6.65</td>
</tr>
</tbody>
</table>

*One patient had a deleterious mutation in both BRCA2 and ATM*
Conclusions on testing in breast cancer

BRCA testing including deletions, Palb2 standard proven utility

Panel testing might identify a mutation in 10% of strong family history with modest unknown value

Most patients will have some unknown variant at this time
Germline Analysis of 12 DNA Repair Genes in Women with Ovarian Cancer

- 360 women unselected for age and family history
  - 273 ovarian, 48 peritoneal, 31 FT, 8 synchronous endometrial & ovarian

- **24%** germline mutation
  - Loss of function
  - >2/3 in BRCA1 or BRCA2
  - 12 genes represented

- Of women with mutation:
  - 30% had no family history
  - 37% ≥ 60 years old at diagnosis

Walsh T et al., PNAS, November 2011:108,10832-18037
Genetic testing in Ovarian Cancer

- When testing for family history that includes ovarian cancer, panel testing may be indicated
- In part due to poor screening option for early detection
- And Effectiveness of prophylactic salpingo-oophorectomy
Resources

- NCCN Guidelines
- ACMG American College of Medical Genetics
- GeneReviews list specific mutation
**BREAST AND OVARIAN MANAGEMENT BASED ON GENETIC TEST RESULTS**

| Intervention Warranted based on gene and/or risk level | Recommend MRI\(^c\)  
(>20% risk of breast cancer\(^d\)) | Recommend RRSO | Discuss Option of RRM |
<table>
<thead>
<tr>
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</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>
<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>
When to consider panel testing

- Consider recommending panel testing: Suspect multiple syndromes, ovarian cancer
- Palb 2 should be included in BRCA testing
- For triple positive; P53, strong breast cancer CHEK 2, ATM so consider panel if including these
- Costs may drive testing, consumer drivers such as Color me
- Patient counselling is complex
- Be prepared for VUS, average 1-2 per test
- Germline testing for targeted therapies
- Somatic testing may also drive germline discovery
Conclusion

- Germline genetic testing is standard of care for high risk individuals when test results will change patient management.
- Best established in breast, ovarian cancer, and Lynch syndrome.
- Panel testing is readily available.
- Panel testing results in identification of additional gene testing when patients undergoing testing are BRCA negative about 10% of the time.
- Risk estimates are likely to be variable based on the clinical context.
- The availability of genetic testing data is being made available of the interpretation data and precise estimates are necessary to identify true clinical value.
- Gene testing of tumors and panel testing also mean an large portion of the population will encounter results they had not expected without counseling. Education and research are key.