The Role of Next Generation Sequencing for Hereditary Cancer Syndromes: A Focus on Endometrial Cancer

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Case Study

• Lisa – 48 year old woman
• Gyn-Onc clinic – 3 weeks of bleeding
• 10 months prior – perforated colonic malignancy at the splenic flexure
• s/p left hemicolecction – pT4 N0
• Adjuvant chemotherapy

Case Study

• CT – “mass arising in the endometrial cavity, infiltrating the myometrium”
• TAH-BSO – pT3a N1 (IIIC) endometrioid carcinoma FIGO grade II with squamous and mucinous differentiation

Overview

• Lynch in endometrial cancer
• Screening methodologies
• Implementation of a NGS panel for hereditary cancer
• Implications and issues of germline testing
  – What to do with unexpected findings?

Deleterious MLH1 mutation detected on sequencing

Lynch Syndrome (aka HNPCC)

- Caused by mutations in 4 mismatch repair genes:
  - MLH1
  - MSH2
  - MSH6
  - PMS2
- EPCAM 3’ deletions → MSH2 promoter methylation and gene silencing
- Autosomal dominant inheritance

Germline mutations in EC

- MSH2 ~ 50-66% (EPCAM)
- MLH1 ~ 24-40%
- MSH6 ~ 10-13% (5 fold greater than CRC)
- PMS2 ~ <5%

Increased Cancer Risks

<table>
<thead>
<tr>
<th>Cancer</th>
<th>Lynch Syndrome</th>
<th>General Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon Cancer</td>
<td>54-74% men</td>
<td>5%</td>
</tr>
<tr>
<td></td>
<td>30-52% women</td>
<td></td>
</tr>
<tr>
<td>Endometrial Cancer</td>
<td>20-60%</td>
<td>2.6%</td>
</tr>
<tr>
<td>Ovarian Cancer</td>
<td>9-12%</td>
<td>&lt;1%</td>
</tr>
<tr>
<td>Gastric Cancer</td>
<td>3-9%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Urinary Tract</td>
<td>8.4% overall,</td>
<td>3%</td>
</tr>
<tr>
<td></td>
<td>up to 27% in</td>
<td></td>
</tr>
<tr>
<td></td>
<td>men</td>
<td></td>
</tr>
<tr>
<td>Small bowel, pancreatic</td>
<td>&lt;4% each</td>
<td>&lt;1% each</td>
</tr>
<tr>
<td>tract, brain</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Lynch – Clinical Criteria

- 1991 – Amsterdam I criteria
- 1996 – Bethesda criteria – who should be tested for MSI
- 1999 – Amsterdam II criteria – increased clinical sensitivity
- 2004 – Revised Bethesda Criteria – Increased sensitivity and establish indications for MSI/IHC testing

Lynch – Clinical Criteria

Revised Bethesda guidelines

REGARDLESS OF AGE OF PATIENT:

- Synchronous, metachronous CRC, or other HNPCC-associated tumors.
- Individual with CRC and at least one first degree relative with CRC/HNPCC tumor less than 50 years of age.
- Patient with CRC in two or more first- or second-degree relatives with CRC/HNPCC-related tumors.
Implications for Patient

- 50% of women with LS will have EC as their sentinel diagnosis
  
  — Critical opportunity to identify LS so that screening for other LS associated cancers can be initiated/modified


DNA Miss Match Repair

Defective Miss Match Repair

- Accumulation of mutations, esp. in microsatellites (MSI)
- Mutations of tumor suppressor genes and oncogenes → tumorigenesis

Microsatellites

- Short tandem repeats
- Mononucleotide
  - AAAAAAAAAAAAA
- Dinucleotide
  - CACACACACACACA
- Trinucleotide
  - CAGCAGCAGCAGCAG
- Prone to slippage during DNA replication

Mismatch Repair Failure Leads to Microsatellite Instability (MSI)

Normal

Microsatellite instability

Expansion (or contraction) of nucleotide repeats
**Clinical significance of MSI**

- CRC
  - better stage-specific prognosis
  - less response to 5-FU
- EC
  - conflicting reports

**Endometrial cancer (EC)**

- MSI also in 17-23% of sporadic EC
  - >70% due to MLH1 methylation
  - Lack BRAF mutations

**MSI-H Histology – Endometrial Cancer**

- Typically endometrioid histology
- Dense peritumoral lymphocytes
- Tumor infiltrating lymphocytes
- Tumor heterogeneity
  - two morphologically distinct tumor populations juxtaposed, each at least 10% of the tumor volume – dedifferentiated adenocarcinoma
- LUS localization
- Associated ovarian clear cell carcinoma

**LS associated EC under-recognized**

- 5 fold increase in MSH6 mutations in EC patients compared to CRC patients (MSI-L or MSS)
- 60-65% of patients >50 yo
- 60-70% did not have a personal or family history
Screening recommendations:
The bottom line
• 25% of individuals with LS don’t meet Amsterdam or Bethesda criteria
• Histology alone is not specific for LS
• Universal screening of newly dx CRC and EC - MMR IHC and MSI testing
• Concordance rates between MSI and IHC are 94% in both CRC and EC.

Patients don’t always know their family history

Possible algorithm

Is Universal Screening cost effective?
• Probably –
  – 6 relatives tested on average per proband identified with LS
  – High Compliance (97%) for cancer surveillance
• Cost analysis not performed for every screening scenario

Lynch Syndrome Testing
• Tumor screening
  – Immunohistochemistry (IHC)
    • Allows for targeted germline testing
  – Microsatellite instability (MSI)
    • Hypermethylation of the promoter - silences transcription of MLH1 (sporadic MSI/loss by IHC)
• Germline Testing
  – Molecular genetic testing of the germline genes MLH1, MSH2, MSH6, PMS2 for a deleterious mutation
IHC patterns

<table>
<thead>
<tr>
<th>Genetic defect</th>
<th>IHC pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MLH1</strong></td>
<td>MLH1 (-/+)* / PMS2 -</td>
</tr>
<tr>
<td><strong>PMS2</strong></td>
<td>MLH1 (+/-) / PMS2 -</td>
</tr>
<tr>
<td><strong>MSH2</strong></td>
<td>MSH2 - / MSH6 -</td>
</tr>
<tr>
<td><strong>MSH6</strong></td>
<td>MSH2 + / MSH6 -</td>
</tr>
</tbody>
</table>

Not all pathogenic mutations result in loss of protein by IHC

*MLH1* gene – False normal
- Some missense mutations
- Certain truncating mutations and large in-frame deletions
- Cases with abnormal methylation

MSI testing

- Surrogate marker of DNA mismatch repair deficiency
- PCR amplification and capillary electrophoresis of mononucleotide microsatellite repeats from tumor and normal
- Compare observed patterns in tumor vs. normal

MSI definition

- NCI Definitions of MSI:
  - **MSI-H**: 2 or more markers showing band shifting
  - **MSI-L**: 1 marker showing band shifting
  - **MSS**: No marker showing band shifting

Microsatellite Analysis:
Mononucleotide repeat contraction

Normal

```
GCTTTAGGAAAAAAAAAAAAAAAAAAAAAAAAATCCCTTAG
CGAAAGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAGGAATC
```

20bp stretch of As

Tumor

```
GCTTTAGGAAAAAAAAAAAAAAAAAAAAAAAAATCCCTTAG
CGAAAGCTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTCAGGAATC
```

15bp stretch of As
Conclusions

• Lynch syndrome is an inherited cancer syndrome with germline mutations of MMR genes
• MSI and MMR IHC are effective screening methods
• Push towards universal screening for CRC and EC patients although the best detection and cost-effective strategy is not yet agreed upon.

Limitations of current screening

• False negative rate 5-10% ~ 33% MLH1 mutations
• Variation in IHC staining patterns
• Cannot identify silencing by methylation
• Cannot identify germline mutation

Role of NGS in screening and diagnosis of LS

Sequencing for screening

Sanger Sequencing
• Single-gene sequencing
• Not cost-effective
• Long turn-around time (gene by gene)

NGS
• Multiple genes
• Multiple patient samples
• More cost-effective
• Improved TAT (sequencing genes in parallel)

NGS options

<table>
<thead>
<tr>
<th></th>
<th>Targeted Gene Panels</th>
<th>Targeted Exome Panel</th>
<th>Whole Exome</th>
<th>Whole Genome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical sensitivity</td>
<td>High</td>
<td>High</td>
<td>Very High</td>
<td>Very High</td>
</tr>
<tr>
<td>Coverage</td>
<td>Complete</td>
<td>Low for some genes</td>
<td>Low for some genes</td>
<td>High</td>
</tr>
<tr>
<td>TAT</td>
<td>Long</td>
<td>Long</td>
<td>Very Long</td>
<td>Very Long</td>
</tr>
<tr>
<td>Cost</td>
<td>High (decreasing)</td>
<td>High</td>
<td>Very High</td>
<td>Very High</td>
</tr>
</tbody>
</table>

NGS targeted panels

• Developed in laboratory
• Prefabricated kit
Focused MMR custom gene panel
PGM - FFPE

<table>
<thead>
<tr>
<th>Gene</th>
<th>Exons</th>
<th>Total Bases</th>
<th>Missed Bases</th>
<th>Total Coverage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MLH1</td>
<td>21</td>
<td>2581</td>
<td>3</td>
<td>99.88</td>
</tr>
<tr>
<td>MSH2</td>
<td>16</td>
<td>2581</td>
<td>217</td>
<td>92.72</td>
</tr>
<tr>
<td>MSH6</td>
<td>10</td>
<td>4193</td>
<td>164</td>
<td>96.09</td>
</tr>
<tr>
<td>FPCAM</td>
<td>9</td>
<td>1044</td>
<td>87</td>
<td>91.67</td>
</tr>
<tr>
<td>PMS2</td>
<td>15</td>
<td>2054</td>
<td>616</td>
<td>78.41</td>
</tr>
</tbody>
</table>

Variants detected

<table>
<thead>
<tr>
<th>Sample</th>
<th>Variants Detected Pre-Filtering</th>
<th>Variants Remaining Post-Filtering</th>
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<tbody>
<tr>
<td>C14</td>
<td>18</td>
<td>10</td>
</tr>
<tr>
<td>C15</td>
<td>16</td>
<td>9</td>
</tr>
<tr>
<td>C16</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>C17</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>C18</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>C19</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>C20</td>
<td>17</td>
<td>9</td>
</tr>
<tr>
<td>C21</td>
<td>18</td>
<td>9</td>
</tr>
<tr>
<td>C22</td>
<td>11</td>
<td>9</td>
</tr>
<tr>
<td>C23</td>
<td>21</td>
<td>9</td>
</tr>
<tr>
<td>C24</td>
<td>19</td>
<td>9</td>
</tr>
<tr>
<td>C25</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>C26</td>
<td>16</td>
<td>9</td>
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<tr>
<td>C27</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>C28</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>C29</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>C30</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>276</td>
<td>9</td>
</tr>
</tbody>
</table>

Known MMR samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Seq. Reading</th>
<th>Read Quality</th>
<th>Variant Coverage</th>
<th>Mismatch identified</th>
<th>Mismatch detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>Sanger</td>
<td>40x</td>
<td>4.3 x 10^6</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C2</td>
<td>Sanger</td>
<td>10x</td>
<td>1.5 x 10^4</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C3</td>
<td>Sanger</td>
<td>10x</td>
<td>1.5 x 10^4</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C4</td>
<td>Sanger</td>
<td>10x</td>
<td>1.5 x 10^4</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>C5</td>
<td>Sanger</td>
<td>10x</td>
<td>1.5 x 10^4</td>
<td>Yes</td>
<td>No</td>
</tr>
</tbody>
</table>

4/5 variants correctly identified by panel
MSH6 variant not detected due to short amplicon fragment size (120 bp)

TruSight Cancer Panel

- Institute of Cancer Research, London
- 94 - cancer predisposition genes (>1,700 exons)
- 284 – SNPs (GWAS)
- Hg19
- Illumina MiSeq instrument

MiSeq Workflow

Validation plan/steps

<table>
<thead>
<tr>
<th>Validation samples</th>
<th>Analytical sensitivity (specificity, PPV/TPR)</th>
<th>Precision</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cell line with MLH1 variant</td>
<td>Minimal specimen requirements</td>
<td>Multiple libraries</td>
<td>Samples confirm</td>
</tr>
<tr>
<td>LS DNA bank samples</td>
<td>Minimal coverage threshold</td>
<td>Same result from same sample</td>
<td></td>
</tr>
<tr>
<td>De-identified banked samples</td>
<td>[Serial dilutions if mixed]</td>
<td>Reproducibility /repeatability (within-run, between-run)</td>
<td></td>
</tr>
</tbody>
</table>
Other considerations

Confirmation
Result Reporting
Informed consent
Return of unexpected findings

Confirmation testing

• Laboratory policy documenting:
  —Indications for confirmatory testing
  —And/or how their assay validation determined that such testing was not required.

Evidence based classification of variants; standardize terminology (draft)

• Pathogenic
• Likely pathogenic
• Benign
• Likely benign
• Uncertain significance

ACMG clinical laboratory standards for next-generation sequencing

Coming soon:

• Standards and Guidelines for the Interpretation of Sequence Variants:
  A Joint Consensus Recommendation of the American College of Medical Genetics and Genomics (ACMG) and the Association of Molecular Pathology (AMP)

Report elements

• Results using HGVS nomenclature
• Interpretation
• References
• Methodology
• Appropriate disclaimers
Return of unexpected findings

ACMG Recommendations for Reporting of Incidental Findings in Clinical Exome and Genome Sequencing

Robert C. Green, MD, MPH,1,2 Jonathan S. Berg, MD, PhD1, Wayne W. Grody, MD, PhD1,4,8 Sarah S. Kitai, PhD, CGC2, Bruce H. Korf, MD, PhD,1,5 Christina L. Martin, PhD, FACMG1, Amy McGuire, JD, PhD1, Elizabeth L. Noebishaw, MS1,6,7, Julianne M. O’Daniel, MS, CGC1, Kelly E. Ormond, MS, CGC1, Heidi R. Rehm, PhD, FACMG1,2,3, Michael S. Watson, MS, PhD, FACMG1,2,3, Marc S. Williams, MD, FACMG1,2,3, and Leslie G. Biossecker, MD1,2,7,

56 gene list (minimum list)

Informed consent

• Consents are variable, some long

• OPT-OUT/OPT-IN

• ACMG recommendations do not allow for any option of not receiving results, regardless of age

Unexpected findings

• Informed consent, including disclosure of policy for handling incidental findings prior to testing, by a genetics professional

• For any germline exome or genome, Laboratory should actively search for variants in the 56 genes (minimum list)

Variants to be reported as incidental findings:

PATHOGENIC = Sequence variation is previously reported and is a recognized cause of the disorder

OR

EXPECTED PATHOGENIC = Sequence variation is previously unreported and is of the type which is expected to cause the disorder

Summary

• Endometrial cancer patients are at risk for LS

• NGS is cost-effective and rapidly incorporated into clinical testing

• NGS Hereditary Cancer Panel institution requires careful consideration and collaboration with clinical colleagues

• Guidelines for NGS clinical testing are now available

DHMC Molecular Pathology Laboratory and Translational Research Program

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Heather Steinmetz
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Terri Wilson
Eric York
Wendy Wells, MD

http://www.lynchscreening.net/
Thank you
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