Genetic Testing for Familial Gastrointestinal Cancer Syndromes

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La Jolla, CA
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The speaker has given a lecture on genetic testing for Ambry Genetics, a commercial genetic testing company, but will provide a balanced overview of an approach to diagnostic testing without recommending any specific vendor.
Familiality and Colorectal Cancer

Colorectal cancer has a high degree of “familiality”

- Sporadic cases
- Some family history (32%)
- Lynch Syndrome (~3%)
- Hamartomatous polyposes (PJS, JPS, HMPS, CD, BRR - rare)
- FAP (~0.5%)
# Genes for Hereditary Colorectal Cancer

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Overview: Genetic testing for Hereditary CRC

• Historically: recognize the phenotype, test for mutations in the gene(s) responsible

• The problems: multiple genes can cause one disease; one gene with clinical pleiotropy; no mutations found for an “obvious” phenotype

• One solution: put multiple genes on a testing panel, and test

• The good news: we found more mutations

• The bad news: challenges in interpretation and using the result
Familial versus Genetic Diseases

• Familial disease
  • multiple cases of a disease in a family
  • could be driven by a single gene
  • could reflect common environmental exposures (diet, tobacco, etc)

• Genetic disease
  • caused by sequence variations in a gene
  • may not cluster in a family because of penetrance, variable expressivity
    • autosomal recessive diseases often (usually) do not cluster in families
Key Genetics Concepts for Diagnosis of fCRC

• **Penetrance**: the proportion of individuals with a given genotype who express the phenotype (such as Lynch syndrome: *MSH2, MLH1 & MSH6* vs *PMS2*), or, FAP vs AFAP

• **Genetic pleiotropy**: multiple phenotypes from mutations in a single gene (such as mutations in *PTEN*)

• **Variable expressivity**: extent of disease expression varies

• **Genetic heterogeneity**: multiple genetic ways to inherit a phenotype, such as Lynch syndrome, FAP (probably all familial GI cancer)
How do we assess possible familial GI cancer?

• Get a good family history
  • Have a mid-level provider get the history and draw a pedigree

• Young people with CRC; people with more than one cancer

• Always consider genetics when you see “polyposis” (>10 polyps in the lifetime)

• Many hospitals screen all CRC for MSI or perform IHC for DNA MMR proteins (*MSH2* and *MSH6*; *MLH1* and *PMS2*)
  • This is to screen for Lynch syndrome
    • but most abnormal tests are **NOT** Lynch syndrome
Gastrointestinal Polyposis

• Consider the total lifetime number of polyps, and the age at diagnosis
  • >100 polyps: classic polyposis
  • 20-99 polyps: oligopolyposis

• The pathology of the polyps is the most important finding
  • Adenomatous polyposis
  • Hamartomatous polyposis
    • Peutz-Jeghers polyps (PJS)
    • Juvenile polyps (JPS)
    • Hyperplastic polyps (could be Cowden’s)
  • Serrated polyps
    • This is usually not familial; linked to no specific genetic locus
Tubulovillous Adenoma
Genes Associated With Adenomatous Polyposis

• **Autosomal dominant**
  • *APC*: causes familial adenomatous polyposis (FAP)
    • Also causes attenuated FAP (AFAP), with many fewer polyps, later onset
    • Note: a deletion of the promoter 1B of *APC* gives classic polyposis, but specific point mutations in this region lead to GAPPS (gastric cancer and proximal gastric polyps)
  • Polymerase proofreading associated polyposis: *POLE & POLD1*
    • Hypermutated tumors

• **Autosomal recessive adenomatous polyposis**
  • *MutYH*-associated polyposis (MAP) – late onset, fewer polyps
  • *NTHL1*-associated polyposis (similar to MAP)
    • *MutYH* and *NTHL1* are both “base excision repair” genes
  • Biallelic DNA mismatch repair deficiency (BMMRD) – childhood cancers
    • Hypermutated tumors, especially CNS; presents with oligopolyposis
    • Biallelic *MSH3* mutations (DNA MMR gene, but this is not BMMRD)
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That’s 5 genes

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That’s 5 genes (not including the MMR genes – 6 more)
Hamartomatous Polyposis Syndromes
Juvenile Polyp
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That’s 5 more (10)
Lynch Syndrome

Hereditary predisposition to colorectal (and other) cancers
- Caused by germline mutations in a DNA mismatch repair (MMR) gene
- Probably the most common heritable cancer disease
- Prevalence is 1/200 - 1/300 in the population
- 3% of all CRCs
- Can be detected by finding MSI or abnormal IHC in tumor

Has multiple “confounders”
- 15% of all CRCs have acquired MMR defect (MLH1)
- Lynch-like syndrome has acquired MMR mutations in tumor
- More (constitutional MLH1 methylation)
Genes that can cause Lynch Syndrome

• *MSH2* (plus silencing via deletions in the 3’ end of *EPCAM*)
• *MSH6* (cancers come on later in life – at “usual” ages for sporadic CRC)

• *MLH1* (most cases of defective function are acquired)
• *PMS2* (low penetrance)

• MSI indicates defective DNA mismatch repair activity
• Abnormal IHC indicates the defective gene (by virtue of *absence* of expression of the protein – and its partner)
• Autosomal dominant inheritance; variable penetrance
• Early-onset cancers; multiple cancers
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**MMR genes: 5 more (15)**
Approaches to Making the Genetic Diagnosis

• Traditional approach (Sanger)
  • Identify the syndrome
  • Test for one gene

• Benefit:
  • Focused, saved resources

• Limitations:
  • Phenotypes not always clear
  • Family history not available
  • Sequential testing not desirable

• Next-generation sequencing
  • Costs of sequencing fell
  • Testing for multiple genes now cheaper than single gene in past

• Benefit:
  • Tests multiple genes at once

• Limitations:
  • Results need confirmation (Sanger)
  • May get uninterpretable results
    • gene “doesn’t fit” your patient’s family
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Possible Results from Genetic Testing

• **POSITIVE** = hereditary cancer susceptibility mutation detected
  • Medical management recommendations made for patient
  • Family members can be tested to see if they carry the mutation as well

• **VARIANT OF UNKNOWN SIGNIFICANCE** = change in gene detected, unclear if it causes increased risk for cancer or not
  • More information needed to classify it definitively
  • More likely to happen the more genes you test
  • Manage patient **ONLY** on basis of personal and family history

• **NEGATIVE** = no hereditary cancer susceptibility mutation detected
  • Does not absolutely mean cancer is not hereditary
  • Does not negate the family history
“Positive for a Deleterious Mutation”

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<th>Interpretation</th>
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<td>APC sequencing</td>
<td>Y1031X (3093T&gt;G)</td>
<td>Deleterious</td>
</tr>
<tr>
<td>comprehensive rearrangement</td>
<td>No Mutation Detected</td>
<td></td>
</tr>
<tr>
<td>G382D (1145G&gt;A) MYH</td>
<td>No Mutation Detected</td>
<td></td>
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<tr>
<td>Y165C (494A&gt;G) MYH</td>
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- Y1031X” indicates codon (amino acid) change
- 3093T>G indicates the nucleotide (DNA) change
Once you know where the mutation is in a family, the test is extremely accurate
# VUS

## Test Results and Interpretation

**GENETIC VARIANT OF UNCERTAIN SIGNIFICANCE**

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<td>E37G (110A&gt;G) (Uncertain)</td>
<td>Uncertain Significance</td>
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<td><em>MSH2</em> sequencing rearrangement analysis</td>
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VUS: the “dreaded” result
Genetic Testing in 2017: Gene Panels

- Individual tests are expensive, and take several weeks to complete
  - If the phenotype is unclear, you may need to order several tests sequentially
- “Next-gen” sequencing makes it possible to get a lot of information faster and quicker
- Genes associated with certain familial cancers are bundled into one large test
  - Often genes for very different diseases are on the same panel (typically breast/ovarian PLUS GI cancer)
Genes on Myriad’s myRisk Panel

- **LS genes**: MSH2, MLH1, PMS2, MSH6, EPCAM
- **Polyposis genes**: APC, MutYH
- **Hamartoma genes**: PTEN, STK11, SMAD4, BMPR1A
- **Breast-ovarian genes**: BRCA1/2, PALB2, CHEK2, BARD1, NBN, BRIP, RAD51C/D, ATM
- **Melanoma-pancreatic cancer**: p16/p14, CDK4
- **And**: p53, CDH1
Genes on Ambry’s 8 Test Panels

• ColoNext: 17 genes (5 Lynch genes plus APC, MutYH, PTEN, SMAD4, BMPR1A, STK11, GREM1, POLE, POLD1; plus p53, CDH1, CHEK2)
• BRCA (2 genes), BRCAplus (6), BRCAplus-Expanded (8), BreastNext (17)
• PancNext (13 genes)
• GYNplus (9 genes: BRCA1/2 and Lynch)
• RenalNext (19 genes)
• OvaNext (24 genes)
• CancerNext (32 genes: BRCA1/2, Lynch, Melanoma, Panc, etc)
• CancerNext-Expanded (49 genes: Colon, Breast, Melanoma, Pancreatic, Gynecologic, and Endocrine)

[Next-generation sequencing; mutations verified by traditional (Sanger) sequencing]
Benefits of Using a Genetic Test Panel

- More likely to get at least one “hit”
- Faster; get all the test results at once
  - Better than sequential testing of genes once/mo
  - A panel can be cheaper than serial testing
  - Helps compensate for erroneous family history
    - Small family size makes it difficult
    - Many people don’t know about cancer diagnoses outside of the immediate family
    - Paternal misattribution
    - Family secrets
Unexpected (positive) Panel Result

SMAD4 mutation detected by a panel (Juvenile Polyposis)
Right Disease, Unexpected Gene

MSH6 mutation detected by a panel; patient has Lynch syndrome
So, what’s the problem with getting a germline diagnosis?

Getting the interpretation right
Which sequence variations contribute to risk?
How do we deal with low-penetrance genes?
Website to Verify Interpretations

- NCBI.NLM.NIH.GOV/CLINVAR
- Go directly, or go through NIH website, click on “All Resources” (left-side menu), ClinVar comes up
- Permits one to type in the exact mutation and verify the interpretation and its provenance
## Interpretation of the Test Results

<table>
<thead>
<tr>
<th>GENE</th>
<th>Pathogenic</th>
<th>Likely pathogenic</th>
<th>VUS (uncertain significance)</th>
<th>Conflicting interpretations</th>
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<td>MSH2</td>
<td>527</td>
<td>112</td>
<td>538</td>
<td>9</td>
</tr>
<tr>
<td>MLH1</td>
<td>539</td>
<td>122</td>
<td>493</td>
<td>6</td>
</tr>
<tr>
<td>MSH6</td>
<td>255</td>
<td>39</td>
<td>632*</td>
<td>13</td>
</tr>
<tr>
<td>PMS2</td>
<td>126</td>
<td>22</td>
<td>307*</td>
<td>13</td>
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<td>165</td>
<td>51</td>
<td>473</td>
<td>46*</td>
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<td>46</td>
<td>13</td>
<td>182</td>
<td>13</td>
</tr>
<tr>
<td>CDH1</td>
<td>51</td>
<td>10</td>
<td>255</td>
<td>20*</td>
</tr>
<tr>
<td>BRCA1</td>
<td>1,149</td>
<td>80</td>
<td>1,344*</td>
<td>211*</td>
</tr>
<tr>
<td>BRCA2</td>
<td>1,255*</td>
<td>75</td>
<td>2,196*</td>
<td>344*</td>
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From NCBI.NLM.NIH.GOV/CLINVAR (Feb, 2016)
Why Bother Testing for Germline Mutations?

• Better management of the patient
  • Follow-up colonoscopy, other cancers (uterine, ovarian, urinary)
  • Getting the surgical management right (extended colectomy)

• Finding asymptomatic affected family members
  • This aspect makes genetic testing cost-effective
  • Typically, only need to test for known familial mutation --- rather than an entire gene panel

• Lynch syndrome CRCs have better natural history, do not respond to cytotoxic adjuvant chemotherapy, and may respond to ICI therapy
  • It is likely that targeted therapies will be developed from this basis
Outcome in MSI CR Cancers

Study of 607 CRCs, all < 50 Y.O.
- 17% MSI-H

Better survival (p<0.001)-76% vs. 54%
- hazard ratio = 0.42

Stage I - 5 year survival - 92% vs. 82%
Stage II - 5 year survival - 92% vs. 77%
Stage III - 5 year survival - 70% vs. 57%

Gryfe et al. NEJM (2000)
The natural history of MSI tumors is better

But… MSI-H CRCs respond less well to 5-FU based chemotherapy

Ribic et al, NEJM, 349:247, 2003 (July 17)
Summary

• There are multiple genetic causes of gastrointestinal cancer
  • Polyposis syndromes
    • familial and non-familial
    • characteristic syndromic clinical features
    • sometimes the phenotype is obvious; often not
  • Non-polyposis familial GI cancer syndromes

• Not all genetic syndromes are necessarily familial
  • Consider in young people with cancer
  • Sporadic polyposis in an isolated patient (recessives, new mutations, etc)
  • MSI in any cancer is suspicious for Lynch syndrome

• Panels are cheaper and more powerful than ever
  • But, you may need help interpreting the results
  • We are still trying to deal with low-penetrance cancer-predispositions of some genes