Defining Lynch Syndrome: Genetic and Phenotypic Heterogeneity

C. Richard Boland, MD
Professor of Medicine
UCSD School of Medicine, San Diego, CA

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Disclosures

• Ambry Genetics: consulting and lectures
Part 1
The evolution in our thinking about familial gastrointestinal cancer
Familial CRC: Polyposis or Non-polyposis?

Polyposis Syndromes

- FAP (dominant)
  - Uncommon: 1/7,000
- Hamartoma Syndromes
  - Rare: PJS, JPS, Cowdens
- Recessives
  - Uncommon: MutYH, NTHL1, MSH3
- oligopolyposis
  - Rare: CMMRD, POLE D/E1

Non-polyposis Syndromes

- Lynch Syndrome
  - (1/226-1/279)
    - (plus Lynch-like syndrome)
- FCC-Syndrome X
  - (less than Lynch syndrome)
- Early-onset CRC (<50)
  - (rising; ~20% is unsuspected LS)

There is some overlap
Aldred Scott Warthin (1866-1931)
First 2 Generations: Cancer Family G

Douglas et al, JAMA 2005
Other reports of familial CRC clusters 1937 to 1967

- Bargen et al. Fam Trends in human cancer. *J. Heredity* 1941 (Mayo Clinic)
- Aure et al. Fam disposition fo cancer of the GI tract. *Acta Chir Scan*, 1964
- Lynch & Krush. Heredity and adenoca of the colon, *Gastroenterology* 1967 (6 families, more data)

Cancer Family G Over the Years

- Warthin’s seamstress’ family (*Arch Int Med*, 1913)
  - “Cancerous fraternities”; this occurred in about 15% of carcinomas
  - Follow-up report by Warthin (*J Cancer Res*, 1925)
  - Another follow-up report by Hauser and Weller (*Am J Cancer*, 1936)

- “Heredity and Adenocarcinoma of the Colon” paper by HT Lynch and Anne J Krush (*Gastro* 1967)

  - 650 members of the family studied; cancers of colon, endometrium, stomach
  - Autosomal dominant inheritance suggested

- Cancer Family G re-revisited Douglas et al. (*JAMA*, 2005)
  - Known family now has 929 members
  - Mutation in *MSH2* (T>G transversion at splice site, exon 4)
  - CRC & EC predominate; falling incidence of gastric cancer over 20th century
Henry T. Lynch, MD (1928-2019)
Lynch Syndrome

- **Lynch Syndrome** = a germline mutation in a DNA MMR gene (1/279)
  - most tumors have characteristic MSI

- **Suspect** with family history, in early-onset cancers, multiple primaries
  - Universal screening of all tumors for MSI or with IHC (MSH2/MSH6, MLH1/PMS2)

- **Confounders of Lynch syndrome (i.e., CRCs with MSI)**
  - acquired methylation of *MLH1* (*BRAF* mut) – 10-12% of all CRC, not familial
  - Lynch-like Syndrome (biallelic somatic mutations of a DNA MMR gene)
  - constitutional (soma-wide) methylation of *MLH1*

- **Familial CRC without MSI**: Familial CRC-type X (familial clusters, not Lynch), oligopolyposis syndromes
Part 2
How was the non-polyposis familial CRC syndrome solved?
Good science and good luck
First discovered by Perucho’s group using AP-PCR

- looking for LOH events in CRC
- found shifted bands (instead of losses)
- recognized these to be deletions in repetitive sequences
- termed them *ubiquitous somatic mutations in simple repeated sequences*

Manuel Perucho, Ph.D. (Burnham Institute, La Jolla, CA)
USM in SRS’s

• A new mechanism for tumor development
  • a novel, “mutator” pathway
• Found USM in SRS’s in 16/137 (12%) of CRCs
  • also present in some adenomas
• Estimated that there were $10^5$ mutations/tumor
• Mutations were all deletions in the simple repeats; losses occurred in the poly-A tails of Alu repeats
• Negatively associated with mutations in p53 and Ras

Microsatellite Instability (MSI)

• Thibodeau et al reported MSI in proximal CRCs (Science 260:816-9, May 7, 1993)

• Coined the term “microsatellite instability” or MIN
  • later, MSI

Stephen Thibodeau, Ph.D., Mayo Clinic
Linking MSI to Lynch Syndrome

International Collaborative Group: Hopkins-Helsinki

“Replicative Error” phenotype (“RER”)

Linked HNPCC to MSI and 2p21 (D2S123)

Peltomaki, et al. – Mapped familial CRC to 2p (D2S123), *Science* 260:812, May 7, 1993
What’s on 2p21?

• Initially, no one knew

• Yeast geneticists (Kolodner, Petes, Liskay) knew that MSI looked like what happened in yeast when the DNA mismatch repair (MMR) genes were knocked out

• Kolodner had just cloned the human *MSH2* gene based upon homology to the yeast gene
May 1993

Who Knew How To Interpret MSI?

Yeast geneticists: knew what MSI meant, but new to human genes

Human Geneticists: linked MSI to LS, but had no idea what MSI meant
Finding the DNA MMR Genes

**MSH2 - 1993**
- Race between Kolodner and Vogelstein-delaChap. for MSH2 (2p)
- Kolodner cloned MSH2 but didn’t find mutations in Lynch families
- Vogelstein: cloned MSH2, found MSH2-LS families; and reported dMMR cell line, HCT116 (H6)
  - *(Cell, Dec 17, 1993)*
- Chan et al: EPCAM *(Nat Gen 2006)*

**MLH1, PMS1, PMS2 – 1994-7**
- Second race for MLH1 (3p)
- Kolodner & Liskay *(Nature Mar 17, 1994)*
- Vogelstein & Finnish colleagues
  - first big bioinformatics success (Venter)
  - *(Science Mar 18, 1994)*
- Two more MutL homologs: PMS1 and PMS2 (Hopkins) *(Nature Sept 1, 1994)*
- Methylation-silencing of MLH1 in sporadic MSI CRC *(Kolodner, Can Res 1997)*
- Finally, MSH6 *(Miyaki Nat Gen Nov, 1997)*
Human MMR System

(a) Mismatch recognition

MutSα

Nascent
Parental

Base–base mispair
1-bp IDL

MutSβ

Nascent
Parental

1-bp IDL
2–4 bp IDL

(b) Recruitment of MLH1–PMS2

Nascent
Parental

MLH1
PMS2

(c) Excision, resynthesis and ligation

TRENDs in Molecular Medicine
Part 3
The clinical and genetic features of Lynch Syndrome
Features of CRCs With MSI

- Proximal colon (70% of Lynch syndrome, and 90% of acquired MSI)
- Often mucinous, poorly differentiated, expanding tumor margin
- Many “tumor infiltrating lymphocytes” (TILs)
  - inflammatory reaction (“Crohn’s-like reaction”), probably due to frame-shift peptides
- Normal LS colon has MMR-deficient crypts (1/cm²)
- Small adenomas (<8 mm) may show MSI/abnormal IHC (43%), but adenomas >10 mm show both abnormalities (100%)
- Adenoma is found adjacent to the CRC in 38% of cases

- Somatic APC mutations in 75% of LS-MSH2, 11% of LS-MLH1
- Somatic β-catenin mutations in 50% of LS-MLH1, 7% of LS-MSH2, 0 in sporadic MSI
- Frequent frameshift mutations in RNF43 (52% adenomas, 58% CRCs)

(Sekine et al, Modern Pathology, 2017; Engel et al, Gastroenterology, April 2020)
Clinical Heterogeneity in Lynch Syndrome

- **Genetic heterogeneity**: multiple genes give rise to a phenotype:
  - e.g., Lynch syndrome: 5 genes produce one disease

- **Penetrance**: the proportion of individuals with a given genotype who express the phenotype
  - varies by gene in LS; lower than initially estimated (before accurate genetic testing)

- **MSH2**: full spectrum of tumors, penetrance for CRC ~35%, any cancer ~70%
- **EPCAM**: 5’ upstream gene to MSH2; loss of termination codon leads to methylation of MSH2 (Alu-rich region)
  - may have a CRC-predominant phenotype if MSH2 is not also affected (i.e., large deletions)
- **MLH1**: full spectrum of tumors, penetrance for CRC ~45%, any cancer ~70%

- **MSH6**: lower penetrance, later onset of CRCs, high penetrance for EC (49% by age 70)
- **PMS2**: much lower penetrance for cancer; CRC <20%; fewer gyn cancers

- Muir-Torre Syndrome: Lynch syndrome plus sebaceous neoplasms of the skin
- Biallelic germline mutations in MMR gene: CMMRD (highly penetrance, pediatric cancers)
Cumulative Incidence of Adenoma in LS (by gene)

Engel et al, *Gastroenterology* 158:1326, April 2020
2747 patients from Germany, Nethlands, Finland
Cumulative Incidence of Advanced Adenoma in LS

Engel et al, Gastroenterology 158:1326, April 2020
Cumulative Incidence of CRC in LS (by gene)

Engel et al, *Gastroenterology* 158:1326, April 2020

\[ P_{\text{overall}} = 0.003 \]

\[ P_{MLH1, MSH2} = 0.468 \text{ (} = 0.137^* \text{)} \]
\[ P_{MLH1, MSH6} = 0.001 \text{ (} < 0.001^* \text{)} \]
\[ P_{MSH2, MSH6} = 0.003 \text{ (} = 0.001^* \text{)} \]

* adjusted for age at index colonoscopy and country
# Cumulative Incidences of Cancer in LS by Gene

<table>
<thead>
<tr>
<th>Gene</th>
<th>CRC Incidence ages 25-70</th>
<th>EC Incidence ages 25-70</th>
<th>Ovarian Cancer Incidence ages 25-70</th>
<th>Any Cancer ages 25-70</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSH2</td>
<td>35%</td>
<td>51%</td>
<td>15%</td>
<td>72%</td>
</tr>
<tr>
<td>MLH1</td>
<td>46%</td>
<td>34%</td>
<td>11%</td>
<td>72%</td>
</tr>
<tr>
<td>MSH6</td>
<td>20%</td>
<td>49%</td>
<td>0</td>
<td>54%</td>
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<tr>
<td>PMS2</td>
<td>10%</td>
<td>24%</td>
<td>0</td>
<td>18%</td>
</tr>
</tbody>
</table>

Prospective study of 1942 LS patients without prior cancer (Mallorca group), Moller et al, *Gut*, 2015
CRCs with MSI have better outcomes

Study of 607 CRCs, all < 50 y.o.
  - 17% MSI-H (presumably, mostly LS)

Better overall survival: 76% vs. 54% (p<.001)
  - hazard ratio = 0.42 (CRC with MSI)

Stage I - 5 year survival - 92% vs. 82%
Stage II - 5 year survival - 92% vs. 77%
Stage III - 5 year survival - 70% vs. 57%
Genetic Analysis Stratifies Outcomes in CRC

Type 1: MSI and BRAF mutation (Methylation of MLH1)

Type 5: MSI, no BRAF or KRAS mut. (Lynch Syndrome)

Type 4: No BRAF, KRAS or MSI

Type 3: KRAS+, no MSI

Type 2: BRAF+, no MSI

All MSIs have better outcomes

Phipps, Gastroenterol 148:77-87, 2015
Approaches to Making the Genetic Diagnosis in familial gastrointestinal cancer syndromes

• Pre-panel approach
  • Identify the syndrome
  • Test for one gene

  • Benefit:
    • Focused, saved resources

  • Limitations:
    • Phenotypes not always clear
    • Sequential testing not desirable
    • Tedious and expensive

• Next-generation sequencing (NGS) panels
  • Costs of sequencing fell dramatically
  • Testing for multiple genes now cheaper than single gene in past
  • Quicker (2 week turn-around)

  • Benefit:
    • Tests multiple genes at once

  • Limitations:
    • May get uninterpretable results
      • gene “doesn’t fit” your patient’s family
Population Prevalence of Lynch Syndrome

• An early guess ~2000: 1/350
  • (5% of population gets CRC, 3% are LS, penetrance ~50% = 1/333)

• Iceland: imputational genetics 1/226
  • Important founder mutations in MSH6 & PMS2

• Australia, US, Canada: 1/279 (A. Win, CEBP, 2017)
  • MLH1 1/1946
  • MSH2 1/2841
  • MSH6 1/758
  • PMS2 1/714
What if you did genetics tests on everyone with CRC?

• Clinic-based CRC patients tested for genetic syndromes
  • N = 1058 subjects tested on 25 gene panel (Dana Farber Cancer Inst)
  • Not selected for possible genetic syndrome (age, FH, MSI)

• 105 (9.9%) carried one or more pathogenic germline mutations
  • 33 (3.1%) Lynch Syndrome (97% had MSI in the tumor)
  • 74 (7.0%) had a non-LS mutation, with 23 (2.2%) in high penetrance cancer genes
    • Included APC (5), MutYH (3), BRCA1/2 (11), PALB2 (2), CDKN2A, p53
  • 36 (3.6%) had lower penetrance mutations
    • Monoallelic MutYH (19), APC*I1307K (17), CHEK2 (2)

• Neither family history nor age was helpful predicting mutations

Yurgelun et al, JCO 35:1086, 2017
General Care of Patients with Lynch Syndrome

• Make a definitive genetic diagnosis, and test FDRs for the mutation
• Annual colonoscopy starting at age 25
• Women should be offered TAH-BSO after completing family
  • screening for endometrial and ovarian cancer prior to surgery is not effective
• Annual urinalysis (risk of TCC of renal collecting system and bladder)
• EGD once, age 35-45, look for H. pylori
  • no need for annual EGDs in western populations
• Dermatological referral for MTS (9% of all LS patients, 28% of LS families)
• Annual mammography in women at age 40 (i.e., standard care)
• No need for routine: EUS (pancreas), CT/MRI of CNS.
Non-CRCs in Lynch Syndrome

- Endometrial cancer: ~40% of women (25% of these before 40 years old)
- Ovarian cancer: 7% (greatest risk ages 40-55)
- Urologic cancers: TCCs of the renal collecting system
  - >8%, especially in men, MSH2
- Pancreatic cancer 1.2% by age 50, 3.7% by age 70
- Breast Cancer 5.4% in LS; no increase in risk, but:
  - ~50% of those in patients <50 yo have defective DNA MMR
- Small intestinal cancers (most often in the duodenum)
  - distal tumors unusual, but very rare outside of familial syndromes
- Prostate: probably increased; 30% by age 70 (?)
- CNS cancers (2.5% MSH2, 1.7% MLH1) – gliomas and GBMs

Small intestine: ten Kate, Gut, 2007; Prostate: Grindedal, CEPB, 2009;
Other Management Issues in LS

• Subtotal colectomy is recommended when the diagnosis of CRC is made
  • prophylactic colectomies not recommended

• Not candidates for 5-FU *adjuvant* chemotherapy (Stage II or III)

• Aspirin (600 mg/day) reduces LS-type cancers by 40%

• Excellent candidates for immune checkpoint therapies
Part 4
The implications of a hypermutated cancer
What is different about CRCs with MSI?

- Colorectal cancers with MSI are different biologically
  - grow faster (hypermutated)
  - less likely to metastasize early
    - HLA Class 1 defects + β2-microglobulin mutations
    - permits immune evasion in the primary tumor mass, but not in circulation
    - metastasis is most often local invasion
  - don’t respond to some chemotherapeutic regimens
  - CRCs with MSI are immunogenic
    - generate antigenic frameshift peptides
    - attract tumor-infiltrating lymphocytes
    - may respond to immune-checkpoint therapies
Comprehensive molecular characterization of human colon and rectal cancer

The Cancer Genome Atlas Network*

To characterize somatic alterations in colorectal carcinoma, we conducted a genome-scale analysis of 276 samples, analysing exome sequence, DNA copy number, promoter methylation and messenger RNA and microRNA expression. A subset of these samples (97) underwent low-depth-of-coverage whole-genome sequencing. In total, 16% of colorectal carcinomas were found to be hypermutated: three-quarters of these had the expected high microsatellite instability, usually with hypermethylation and MLH1 silencing, and one-quarter had somatic mismatch-repair gene and polymerase ε (POLE) mutations. Excluding the hypermutated cancers, colon and rectum cancers were found to have considerably similar patterns of genomic alteration. Twenty-four genes were significantly mutated, and in addition to the expected APC, TP53, SMAD4, PIK3CA and KRAS mutations, we found frequent mutations in ARIDIA, SOX9 and FAM123B. Recurrent copy-number alterations include potentially drug-targetable amplifications.
Hypermutated CRCs Are Unique

Somatic mutations in CRCs - 2 clusters

A different cluster of genes are mutated in hypermutated tumors (only APC in both lists)

Most sporadic tumors: our old friends (from 1990): APC, p53, KRAS

MMR deficiency predicts PD-1 Blockade response

• 86 patients, advanced tumors of 12 sites in 20 wk anti-PD-1 study
  • CRC and other GI, endometrial, neuroendocrine, prostate, thyroid
  • Treated with pembrolizumab (74% had AEs, mostly low-grade)

• Complete response: 21%
• Partial response: 33% (objective responses in 53%)
• Stable disease: 23%
• Progressive disease: 14%

Dung Le: Science 357:409, July 28, 2017
Thank you
crboland@health.ucsd.edu