Prevalence of germline cancer susceptibility gene mutations in a consecutive series of colorectal cancer patients

Matthew B. Yurgelun¹,²,³, Brian Allen⁴, Lauren K. Brais¹, Philip G. McNamara¹, Chinedu I. Ukaegbu¹, Hajime Uno¹,³, John Kidd⁴, Nanda Singh⁴, Jason L. Hornick²,³, Richard J. Wenstrup⁴, Charles S. Fuchs¹,²,³, Matthew H. Kulke¹,²,³, Anne-Renee Hartman⁴, Sapna Syngal¹,²,³

¹Dana-Farber Cancer Institute, Boston, MA
²Brigham & Women’s Hospital, Boston, MA
³Harvard Medical School, Boston MA
⁴Myriad Genetic Laboratories, Inc., Salt Lake City, UT
Background

• Hereditary factors play a key role in colorectal cancer (CRC) risk

• Population-based studies have attempted to estimate prevalence of hereditary CRC syndromes by performing germline testing on CRC patients with particular high-risk features:

  – 2-4% prevalence of Lynch syndrome among CRC patients when universal MSI and/or mismatch repair immunohistochemistry (MMR IHC) testing used to guide germline testing

  – <1% combined prevalence of germline APC and biallelic MUTYH mutations among CRC patients, using pre-selection for polyposis phenotypes

  – Prevalence of other hereditary syndromes in CRC patients unknown (presumed to be rare)

Presented by: Matthew B. Yurgelun, MD

Prior studies of multigene panel testing in patients with suspected hereditary CRC have shown surprisingly high frequencies of unexpected germline mutations:

- Cragun, et al: Among 586 patients undergoing clinical panel testing for hereditary CRC syndrome, 29% of patients who had “actionable” mutations did not fulfill clinical criteria for their syndrome
  - 1/3 Lynch syndrome, 1/3 FAP/MAP, 1/3 other
- Yurgelun, et al: Among 1260 patients with suspected Lynch syndrome, multigene panel testing showed 39% of all mutations found were in non-Lynch genes
  - 1.2% of overall cohort had BRCA1/2 mutation, though most did not meet clinical criteria for BRCA1/2 testing

Limitations of these studies → Ongoing uncertainty about the utility of panel testing:

- Laboratory-based cohorts, lack of MSI and MMR IHC data, unable to verify clinical histories
  - Do patients with unexpected mutations have atypical phenotypes for their syndrome, or were they just clinically misclassified?
- Studies of patients with high-risk features (age, personal/family of cancer)
  - What is the full spectrum of inherited cancer susceptibility in unselected patient cohorts?
Aim

• To determine the prevalence of germline cancer susceptibility gene mutations among colorectal cancer patients without selection for high-risk features (e.g. age at diagnosis, personal/family history, or MMR-D / MSI-H tumor testing results)
Methods

• Individuals age ≥18 seen at the Dana-Farber Cancer Institute for CRC diagnosis recruited to sample registry

• Study population: 1100 consecutively ascertained participants who provided a blood sample and were enrolled from 12/2008 – 3/2014

• Frozen whole blood sent to commercial laboratory (Myriad Genetic Laboratories, Inc.) for testing with a 25-gene hereditary cancer panel
  – All sequence variations classified for pathogenicity:
    • Deleterious
    • Suspected deleterious
    • Variant of uncertain significance (VUS)
    • Favor polymorphism
    • Polymorphism

• Approved by Dana-Farber/Harvard Cancer Center Institutional Review Board
# Cancer susceptibility genes on a 25-gene panel

## High-penetrance

- Lynch syndrome
  - MLH1*, MSH2*, MSH6*, PMS2*, EPCAM*
- Adenomatous polyposis syndromes
  - APC*, MUTYH* (biallelic)
- Hamartomatous polyposis syndromes
  - BMPR1A*, PTEN*, SMAD4*, STK11*
- Familial atypical multiple mole melanoma syndrome
  - CDKN2A
- Li-Fraumeni syndrome
  - TP53*
- Hereditary diffuse gastric cancer
  - CDH1*
- Hereditary breast/ovarian cancer
  - BRCA1*, BRCA2*
- Hereditary breast/pancreatic cancer
  - PALB2*

## Moderate-penetrance

- Linked to CRC risk
  - APC*I1307K
  - CHEK2*
  - MUTYH (monoallelic)
- Not linked to CRC risk
  - ATM*
  - BARD1
  - BRIP1*
  - CDK4
  - NBN
  - RAD51C*
  - RAD51D*

* Indicates genes for which NCCN guidelines recommend specialized screening/risk-reducing interventions
Clinical data – Medical Record Review

- Demographics
- Personal medical history, including results of prior genetic testing
- Family history of cancer
- Details of CRC diagnosis, including confirmation of diagnosis
  - Age at 1st CRC diagnosis
  - Stage
  - Location
  - # prior CRC diagnoses
  - \textit{KRAS} / \textit{NRAS} and \textit{BRAF} mutation analysis
  - Microsatellite instability (MSI) and mismatch repair immunohistochemistry (MMR IHC) results

- \textit{PREMM}_{1,2,6} scores calculated (http://premm.dfci.harvard.edu) for all participants, using data on personal and family histories of cancer
Study Population

• 8 excluded due to pathology review revealing non-CRC diagnosis
  – 6 with anorectal SCC
  – 1 cholangiocarcinoma
  – 1 Müllerian adenocarcinoma

• 33 excluded due to failed germline testing

→ Final study population N=1059
### Clinical features of cohort (N=1059)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N (%)</th>
<th>Characteristic</th>
<th>N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/Female</td>
<td>588 (56%) / 471 (44%)</td>
<td>Right-sided CRC</td>
<td>353 (33%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Left-sided CRC</td>
<td>362 (34%)</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>940 (89%)</td>
<td>Rectal/rectosigmoid CRC</td>
<td>342 (32%)</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>50 (5%)</td>
<td>Personal history of &gt;1 CRC</td>
<td>29 (3%)</td>
</tr>
<tr>
<td>Hispanic/Latino</td>
<td>27 (3%)</td>
<td>Personal history of other non-CRC cancer†</td>
<td>160 (15%)</td>
</tr>
<tr>
<td>Asian</td>
<td>22 (2%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median age at 1st CRC, years</td>
<td>55.7 (SD ±12.6) range 21-92</td>
<td>Family history any cancer</td>
<td>871 (82%)</td>
</tr>
<tr>
<td>Age &lt;50 years</td>
<td>337 (32%)</td>
<td>Family history CRC (any)</td>
<td>337 (32%)</td>
</tr>
<tr>
<td>Stage 0/I</td>
<td>130 (12%)</td>
<td>Family history CRC (1st degree relative)</td>
<td>138 (13%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>202 (19%)</td>
<td>Family history breast cancer</td>
<td>285 (27%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>404 (38%)</td>
<td>Median PREMM_{1,2,6} score (IQR)</td>
<td>3.93% (2.58-6.67)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>321 (30%)</td>
<td>PREMM_{1,2,6} score ≥5%</td>
<td>415 (39%)</td>
</tr>
</tbody>
</table>

*Excluding cutaneous basal / squamous cell carcinomas*
Tumor testing results (N=1059)

- **MSI / MMR IHC testing**: 566 (53%) with results
  - 85% MSS / MMR-P
  - 12% MSI-H and/or MMR-D
  - 2% MSI-L / MMR-P

- **KRAS / NRAS mutation status**: 741 (70%) with results
  - 57% wildtype
  - 43% mutation

- **BRAF mutation status**: 648 (61%) with results
  - 93% wildtype
  - 7% mutation
Germline testing results

- 106 / 1059 (10.0%; 95% CI 8.3-12.0%) with ≥1 pathogenic germline mutation(s)
  - 70 (6.6%) with mutations in genes for which NCCN guidelines recommend specific screening and/or risk-reducing intervention
  - 33 (3.1%) patients with Lynch syndrome mutations
  - 75 (7.1%) patients with non-Lynch syndrome mutations
    - 2 patients with both a Lynch and non-Lynch mutation
    - 3 patients with two non-Lynch mutations

- 416 VUS detected among 336 patients (31.8% of cohort)
  - Most common: ATM (52), APC (36), PMS2 (34), NBN (32)
Pathogenic mutations identified by a 25-gene hereditary cancer panel among 1059 consecutive patients with CRC

- No germline mutation: 90.0%
- Moderate-penetrance mutations: 4.8%
- High-penetrance mutations*: 5.2%

* Includes 4 patients with both a high- and a moderate-penetrance mutation
Pathogenic mutations identified by a 25-gene hereditary cancer panel among 1059 consecutive patients with CRC

Moderation-penetrance mutations: 4.8%
High-penetrance mutations*: 5.2%

* Includes 4 patients with both a high- and a moderate-penetrance mutation
Pathogenic mutations identified by a 25-gene hereditary cancer panel among 1059 consecutive patients with CRC

- High-penetrance mutations* (5.2%)
- Moderate-penetrance mutations (4.8%)

* Includes 4 patients with both a high- and a moderate-penetrance mutation
Pathogenic mutations identified by a 25-gene hereditary cancer panel among 1059 consecutive patients with CRC

Lynch syndrome** (3.1%)
- MLH1 (N=13)
- MSH2 (N=7)
- MSH6 (N=6)
- PMS2 (N=7)

Polyposis mutations (0.8%)
- APC (N=5)
- biallelic MUTYH (N=3)

Other high-penetrance mutations (0.4%)
- PALB2 (N=2)
- CDKN2A (N=1)
- TP53 (N=1)

BRCA1/2 (0.9%)
- BRCA1 (N=3)
- BRCA2 (N=7)

** Includes 1 patient with both an MLH1 and BRCA2 mutation
Pathogenic mutations identified by a 25-gene hereditary cancer panel among 1059 consecutive patients with CRC

Moderate-penetrance CRC gene mutations (3.3%)
- monoallelic MUTYH (N=18)
- APC*1307K (N=14)
- CHEK2 (N=3)

High-penetrance mutations* (5.2%)
- ATM (N=10)
- BARD1 (N=1)
- BRIP1 (N=3)
- NBN (N=2)

Other moderate-penetrance mutations (1.5%)
- MUTYH (N=18)
- APC*1307K (N=14)
- CHEK2 (N=3)

* Includes 4 patients with both a high- and a moderate-penetrance mutation
Lynch syndrome

- 3.1% of overall cohort

- 28/29 (97%) had tumors with MSI-H and/or MMR-D
  - 4 had missing MSI / MMR IHC data

- 28/33 (85%) had PREMM1,2,6 scores ≥5%

- 32/33 (97%) met NCCN criteria for Lynch syndrome evaluation
Non-Lynch MSI-H / MMR-D Colorectal Cancers

- 42 patients with MSI-H / MMR-D CRCs lacked a germline Lynch mutation
  - 19 presumed sporadic
    - 14 with somatic *BRAF* mutations
    - 5 with somatic *MLH1* promoter hypermethylation
  - 23 lacked confirmed germline or sporadic etiology
    - 6 with germline VUS in MMR gene that corresponded to pattern of MMR protein loss by IHC
    - 17 (1.6% of cohort) with unexplained MSI-H / MMR-D
      - “Lynch-like syndrome”
      - Median age at CRC: 47 years (range 21-78)
      - 9/17 (53%) with family history of CRC
High-penetrance non-Lynch mutations (N=23)

- **BRCA1/2 (N=11)** – 1.0% of cohort (most common non-Lynch high-penetrance finding)
  - 8 (73%) failed to meet NCCN criteria for BRCA1/2 testing
    - 2 (18%) with personal history of BRCA-associated cancer (breast cancer and melanoma)
    - 5 (45%) with family history of breast cancer, 1 (9%) ovarian cancer
  - 5 (45%) with CRC at age <50 (range 31-69 years)
  - 3 (27%) Ashkenazi founder mutations

- Polyposis genes: **APC (N=5)** and biallelic **MUTYH (N=3)** – 0.8% of cohort
  - 3 (38%) failed to meet NCCN criteria for polyposis genetic testing (<20 lifetime adenomas)
  - Median age at 1st CRC diagnosis: 45.5 years (range 25-62); 3 (38%) with CRC at age ≥50

- Other high-penetrance genes: **PALB2 (N=2)**, **CDKN2A (N=1)**, **TP53 (N=1)** – 0.4% of cohort
  - None had personal or family histories suggestive of their respective syndrome
  - Median age at 1st CRC diagnosis: 65.5 years (range 64-72)

- Overall, 8/23 (35%) had clinical histories suggestive of their syndrome
Moderate-penetrance mutations (N=55)

- Linked to CRC risk (N=39) – monoallelic MUTYH (19), APC*I1307K (17), CHEK2 (3)
  - 4 also had high-penetrance mutations (2 BRCA1 and APC*I1307K, 1 BRCA2 and monoallelic MUTYH, 1 MSH2 and APC*I1307K)
  - Median age at 1st CRC† diagnosis: 59 years (range 40-82)

- Not linked to CRC risk (N=16) – ATM (10), BARD1 (1), BRIP1 (3), NBN (2)
  - Median age at 1st CRC† diagnosis: 51 years (range 37-76)

† Excluding those with both a moderate- and high-penetrance mutation
# Multivariable analysis

Factors associated with presence of a non-Lynch mutation (versus non-mutation carriers)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Odds ratio (95% confidence interval)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1 CRC diagnosis (reference: 1 CRC diagnosis)</td>
<td>3.94 (1.38, 11.22)</td>
</tr>
<tr>
<td>Personal history of non-CRC cancer diagnosis†</td>
<td>1.80 (0.98, 3.29)</td>
</tr>
<tr>
<td># 1\textsuperscript{st}/2\textsuperscript{nd} degree relatives with CRC (reference: 0 relatives)</td>
<td>1 relative: 0.98 (0.53, 1.84)  \geq2 relatives: 1.50 (0.61, 3.73)</td>
</tr>
<tr>
<td>Family history ovarian cancer</td>
<td>2.86 (1.26, 6.52)</td>
</tr>
<tr>
<td>CRC stage (reference: Stage 0/I)</td>
<td>Stage II/III: 0.38 (0.19, 0.77) Stage IV: 0.66 (0.32, 1.37)</td>
</tr>
<tr>
<td>KRAS mutation status</td>
<td>KRAS G12C mutation: 5.26 (2.06, 13.43) Unknown KRAS status: 0.81 (0.45, 1.46) Other KRAS status: 1.0 (reference)</td>
</tr>
</tbody>
</table>

† Excluding cutaneous basal / squamous cell carcinomas

Presented by: Matthew B. Yurgelun, MD
KRAS G12C (c.34G>T) mutations

- Uncommon mutation (found in approx 3% of all CRCs)

- Prior studies have shown significant association with MUTYH-associated polyposis
  - MUTYH mutations lead to defective base excision repair, which leads to accumulation of somatic G:C>T:A transversions

- In our cohort (among patients with known RAS status):
  - 2 / 2 (100%) patients with biallelic MUTYH mutations
  - 2 / 14 (14%) patients with monoallelic MUTYH mutations
    - Suggests possible defective base excision repair defects from monoallelic MUTYH mutation
  - 20 / 668 (3%) of mutation-negative patients (P<0.001)
Summary

- Germline cancer susceptibility gene mutations found in 10.0% of consecutive CRC patients
  - Overall, 6.6% with mutations in genes for which NCCN guidelines recommend specific management

- 5.2% with mutations in high-penetrance genes
  - 3.1% with Lynch syndrome
    - Virtually all detected by universal MSI / MMR IHC tumor testing and PREMM$^{1,2,6}$ analysis
    - Consistent with prior population-based studies that used universal MSI / MMR IHC pre-screening
  - 1.0% with $BRCA1/2$ mutations $\Rightarrow$ only 27% met NCCN criteria for $BRCA1/2$ testing
  - 0.8% with polyposis mutations ($APC$ or biallelic $MUTYH$)

- Only 35% of high-penetrance non-Lynch mutation carriers had clinical histories suggestive of their syndrome

- Unclear how best to identify non-Lynch mutation carriers
  - $KRAS$ G12C mutations predict for biallelic $MUTYH$ mutations (and possibly monoallelic $MUTYH$ mutations)
  - Personal history of $>1$ CRC significantly associated with having a non-Lynch mutation (compared to mutation-negative individuals), but this has a low sensitivity for detecting mutation carriers
Strengths and Limitations

**Strengths**

- Large, consecutive cohort without preselection for high-risk features
- Extensive clinical data
  - Personal history
  - Family history
  - Tumor testing results
- Testing done through CLIA-approved commercial laboratory
  - Extensive experience in variant classification

**Limitations**

- Clinic-based cohort
- Some details limited
  - Incomplete tumor testing data
    - Many patients enrolled prior to universal MMR IHC testing
  - Lack of data on Ashkenazi ancestry
  - Incomplete data on polyp #/histology
  - Unable to verify personal/family history data beyond medical record review

Presented by: Matthew B. Yurgelun, MD
Conclusions (1)

• Universal MSI / MMR IHC and PREMM\textsubscript{1,2,6} testing identifies almost all Lynch syndrome probands
  – Substantial fraction of patients with unexplained MSI-H / MMR-D CRCs

• Spectrum of genetic factors in CRC patients is more diverse than traditionally appreciated.
  – Many mutation carriers lack classic clinical histories for their specific syndrome

• Moderate-penetrance genes / alleles (monoallelic \textit{MUTYH} mutations, \textit{APC}^*\textit{I1307K}) are relatively common, though significance remains unclear

• Need to define the full spectrum of cancer risk associated with high-penetrance susceptibility genes: e.g. \textit{BRCA1/2}, \textit{PALB2}, \textit{TP53}
Conclusions (2)

• Traditional thinking is that BRCA1/2 mutations do not confer CRC risk

• BRCA1/2 mutations continue to be identified in a subset of patients with CRC who lack classic hereditary breast/ovarian cancer histories
  – 1.0% in consecutive CRC patients
  – 1.2% of patients\(^1\) with suspected Lynch syndrome
  – Higher than expected (0.2-0.3\(\%\))\(^2\) population prevalence
  – More common than polyposis syndromes in these studies