Lynch syndrome screening in colorectal cancers

10 October 2015
Masato Yozu
Middlemore Hospital, Auckland, New Zealand
Lynch syndrome screening

**Colonoscopy: Biopsy for ‘mass’ lesion**

- **Diagnosis of cancer/adenomatous mucosa**
  - No Loss of MMR (Lynch unlikely)
  - Loss of MSH2+MSH6
  - Loss of MSH6
  - Loss of PMS2 (Likely Lynch)
    - NZFGICS
  - Loss of MLH1+PMS2
    - BRAF mutation analysis on biopsy (PCR)
    - BRAF/KRAS/NRAS if metastatic disease (Mass array)
    - BRAF mutated (Lynch unlikely)
      - NZFGICS
    - BRAF wild type (Possibility of Lynch)

**Surgical resection of the tumour**

- Repeat MMR, IHC and BRAF if inconclusive on biopsy specimen
B.B. 71y F

- Presented with epigastric pain and elevated tumour markers (tested by GP): CA125 119, CEA 22
- CT:
  - Liver ill-defined mass 9cm ?primary ?metastasis
  - Left adrenal lesion 1.6cm, suspicious for metastasis
  - Omental nodules, suspicious for metastasis
- Colonoscopy: Mid ascending colon mass
- PMHx:
  - Ovarian cancer at age 55y (UK)
- FHx:
  - Not documented
Biopsy ascending colon mass

- Adenocarcinoma, low grade
Biopsy ascending colon mass
MLH1
Biopsy ascending colon mass
PMS2
Biopsy ascending colon mass

MSH2
Biopsy ascending colon mass
MSH6
Biopsy ascending colon mass

- Adenocarcinoma, low grade
  - MLH1: Loss
  - PMS2: Loss
  - MSH2: Focal loss (abnormal)
  - MSH6: Focal loss (abnormal)
- Molecular analysis (mass array)
  - BRAF: Negative
  - KRAS: Positive (G12D)
  - NRAS: Negative
Discussion

B.B. 71y F

• Assess BRAF, KRAS and NRAS mutation in clinical stage 4
• Biopsy is the only tissue available for ancillary studies.
MMR deficiency in CRCs

• 10-15% of all CRCs are MMR deficient
• 70% of MMR deficient CRCs are sporadic
• The rest are Lynch-like (60%) or Lynch syndrome (40%)
Sporadic vs Lynch syndrome

- Sporadic MLH1 loss (MLH1 promoter methylation)
  - Older age (generally)
  - No significant past medical history (generally)
  - No significant family history (generally)
  - BRAFV600E mutation mostly present (75-80%)
  - MLH1 promoter methylation mostly present (75-80%)

Note: None of above is 100% specific
Determining the ‘likelihood’ of Lynch syndrome

• Any MMR IHC loss involving MLH1
  – Age
  – Past medical history
  – Family history
  – MMR IHC/MSI
  – BRAF mutation analysis
  – MLH1 promoter methylation analysis

• Diagnosis of Lynch syndrome is only made by detection of pathogenic MMR gene mutation
Q.1 : MSI vs IHC
Identifying Lynch syndrome: MSI or IHC?

(Gastroenterology 2015;149:783-813)
Identifying Lynch syndrome

MSI or IHC?

IHC

(Gastroenterology 2015;149:783-813)
Identifying Lynch syndrome: MSI or IHC?

MSI+IHC

(Gastroenterology 2015;149:783-813)
Q.2 : BRAF vs Methylation study
Identifying sporadic cases
MLH1 promoter methylation vs BRAF mutation

MLH1 promoter methylation

(Gastroenterology 2015;149:783-813)

<table>
<thead>
<tr>
<th>Study</th>
<th>TP</th>
<th>FP</th>
<th>FN</th>
<th>TN</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bergino 2009</td>
<td>20</td>
<td>1</td>
<td>16</td>
<td>6</td>
<td>0.56 [0.38, 0.72]</td>
<td>0.86 [0.42, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bouzourene 2010</td>
<td>11</td>
<td>1</td>
<td>10</td>
<td>15</td>
<td>1.00 [0.72, 1.00]</td>
<td>0.94 [0.70, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Canard 2012</td>
<td>43</td>
<td>0</td>
<td>18</td>
<td>4</td>
<td>0.70 [0.57, 0.81]</td>
<td>1.00 [0.40, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ewald 2007</td>
<td>11</td>
<td>0</td>
<td>5</td>
<td>4</td>
<td>0.69 [0.41, 0.89]</td>
<td>1.00 [0.40, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gausachs 2012</td>
<td>16</td>
<td>1</td>
<td>31</td>
<td>23</td>
<td>0.34 [0.21, 0.49]</td>
<td>0.96 [0.79, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Julie 2008</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0.91 [0.59, 1.00]</td>
<td>1.00 [0.16, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuismanen 2000</td>
<td>30</td>
<td>12</td>
<td>6</td>
<td>14</td>
<td>0.83 [0.67, 0.94]</td>
<td>0.54 [0.33, 0.73]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lubomierski 2005</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>1</td>
<td>1.00 [0.29, 1.00]</td>
<td>0.25 [0.01, 0.81]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>McGivern 2004</td>
<td>18</td>
<td>2</td>
<td>3</td>
<td>7</td>
<td>0.86 [0.64, 0.97]</td>
<td>0.78 [0.40, 0.97]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perez-Carbonell 2010</td>
<td>49</td>
<td>0</td>
<td>14</td>
<td>10</td>
<td>0.78 [0.66, 0.87]</td>
<td>1.00 [0.69, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rahner 2008</td>
<td>5</td>
<td>3</td>
<td>20</td>
<td>57</td>
<td>0.20 [0.07, 0.41]</td>
<td>0.95 [0.86, 0.99]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toon 2013</td>
<td>11</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>0.85 [0.55, 0.98]</td>
<td>1.00 [0.54, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wheeler 2000</td>
<td>7</td>
<td>0</td>
<td>3</td>
<td>10</td>
<td>0.70 [0.35, 0.93]</td>
<td>1.00 [0.69, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yamamoto 2012</td>
<td>32</td>
<td>0</td>
<td>8</td>
<td>30</td>
<td>0.80 [0.64, 0.91]</td>
<td>1.00 [0.88, 1.00]</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Pooled sensitivity</strong></td>
<td><strong>0.75 (0.59; 0.86)</strong></td>
<td><strong>0.94 (0.79; 0.98)</strong></td>
<td><strong>0.75 (0.59; 0.86)</strong></td>
<td><strong>0.94 (0.79; 0.98)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Identifying sporadic cases
MLH1 promoter methylation vs BRAF mutation

(BRAF)

(Gastroenterology 2015;149:783-813)
Important points of MLH1 promoter methylation and BRAF testing

• Neither methods are perfectly specific
  – Very rarely CRCs in Lynch syndrome can show MLH1 promoter methylation or BRAF mutation

• Neither methods are perfectly sensitive
  – Negative result does not mean the patient has Lynch syndrome

• MLH1 promoter methylation study: ‘methylated’ or ‘unmethylated’ are based on quantitative number of the degree of methylation
  – cut off dependent (cf. BRAF is qualitative)
  – MLH1 was ‘methylated’ in 16% of Lynch syndrome and 92% of sporadic case (Cancer 2015;121:1395-1404)
Q.3 : Biopsy vs Resection
Advantages of MMR/BRAF testing on biopsy specimen

1. Changes in surgical procedures for likely Lynch syndrome patients
   • Total colectomy
   • Prophylactic gynaecological surgery

2. Issues of MMR IHC in surgical specimen
   • False negative staining in poorly fixed specimen

3. No surgical resection in stage IV patients

4. Issues of MMR IHC/MSI in post neoadjuvant therapy specimens
   • False negative staining
   • Aberrant MSH6 nucleolar positivity
   • No tumour/minimal tumour
“To facilitate surgical planning, tumour testing on suspected CRC should be performed on preoperative biopsy specimens, if possible.”

Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer
Gastroenterology 2014;147:502-526
Q.4 : Universal vs Selected screening
<table>
<thead>
<tr>
<th>Table 1. Bethesda Guidelines (Revised)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Colorectal cancer diagnosed before age 50 years</td>
</tr>
<tr>
<td>2. Multiple colorectal cancer or HNPCC-related cancers(^a)</td>
</tr>
<tr>
<td>3. Colorectal cancer with MSI-related histology(^b) diagnosed before age 60 years</td>
</tr>
<tr>
<td>4. Colorectal cancer or HNPCC-related cancer diagnosed in at least one first-degree relative before age 50 years</td>
</tr>
<tr>
<td>5. Colorectal cancer or HNPCC-related cancer diagnosed in at least 2 first- or second-degree relatives at any age</td>
</tr>
</tbody>
</table>

**NOTE.** Any criterion (1–5) can be met.
HNPCC, hereditary nonpolyposis colorectal cancer.
\(^a\)Includes cancer of endometrium, small bowel, pelviureter, biliary tract, stomach, ovary, pancreas, or brain (mainly glioblastoma multiforme).
\(^b\)Tumor-infiltrating lymphocytes, Crohn’s-like lymphocytic reaction, mucin/signet ring cell differentiation, medullary growth pattern.

Note: No recommendations for MSI/MMR testing in patients >60 years of age
Lynch syndrome–associated colorectal carcinoma: frequent involvement of the left colon and rectum and late-onset presentation supports a universal screening approach

Douglas J. Hartman MD, Randall E. Brand MD, Huankai Hu MD, Nathan Bahary MD, PhD, Beth Dudley MS, MPHq3, Simon I. Chiosea MD, Marina N. Nikiforova MD, Reetesh K. Pai MD,*

Department of Pathology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213
Department of Internal Medicine, Division of Gastroenterology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213
Department of Internal Medicine, Division of Medical Oncology, University of Pittsburgh Medical Center, Pittsburgh, PA 15213
Cancer Genetics Program, University of Pittsburgh Medical Center, Pittsburgh, PA 15213

Received 6 May 2013; revised 19 June 2013; accepted 21 June 2013
Consecutive Colorectal Carcinomas evaluated by MSI PCR and/or MMR IHC (N = 1292)

Carcinomas with MSI-H and/or Abnormal MMR IHC Expression (n = 150; 11.6%)

Sporadic MSI-H Colorectal Carcinoma (n = 112; 9.1%)
1. BRAF V600E Positive (n = 91)
2. BRAF wild-type but MLH1 promoter hypermethylation positive or germline mutation negative (n = 19)
3. BRAF wild-type with negative family/personal history and loss of MLH1 and PMS2 expression (n = 2)

LS/Probable LS-associated Colorectal Carcinoma (n = 38; 2.9%)
1. Abnormal MSH2 and/or MSH6 (n = 23)
2. Isolated loss of PMS2 (n = 5)
3. Abnormal MLH1 and PMS2 and Wild-type BRAF (n = 10)
   a) Positive germline MLH1 mutation (n = 2)
   b) Negative MLH1 promoter hypermethylation (n = 8)

Fig. 1  Flowchart detailing the MSI-H colorectal carcinoma study group stratified into LS/ probable LS-associated and sporadic subgroups.
12 out of 38 Lynch syndrome patients (32%) presented >60 years. Not identified with revised Bethesda guidelines.
Prediction models

• MMRpredict model: hnpccpredict.hgu.mrc.ac.uk/
  – sensitivity 69% and specificity 90%
• MMRpro model: www4utsouthwestern.edu/breasthealth/cagene/
  – sensitivity 89% and specificity 85%
• PREMM model: premm.dfc.ihavard.edu.
  – sensitivity 90% and specificity 67%

Note: All require accurate family history
Universal screening vs selected population screening

EGAPP RECOMMENDATION STATEMENT

Recommendations from the EGAPP Working Group: genetic testing strategies in newly diagnosed individuals with colorectal cancer aimed at reducing morbidity and mortality from Lynch syndrome in relatives

Evaluation of Genomic Applications in Practice and Prevention (EGAPP) Working Group*


Summary: Found sufficient evidence that testing for Lynch syndrome in newly diagnosed colorectal cancer patients reduce morbidity and mortality in relatives.
Guideline: Testing for MMR deficiency of newly diagnosed CRC should be performed. This can be done for all CRCs, or CRC diagnosed at age 70 years or younger, and in individuals older than 70 years who have a family history concerning for LS.

Analysis can be done by IHC testing for the MLH1/MSH2/MSH6/PMS2 proteins and/or testing for MSI. Tumors that demonstrate loss of MLH1 should undergo BRAF testing or analysis of MLH1 promoter hypermethylation.
Hereditary Colorectal Cancer Syndromes: American Society of Clinical Oncology Clinical Practice Guideline
Endorsement of the Familial Risk–Colorectal Cancer: European Society for Medical Oncology Clinical Practice Guidelines


- Tumor testing for DNA mismatch repair (MMR) deficiency with immunohistochemistry for MMR proteins and/or MSI should be assessed in all CRC patients. As an alternate strategy, tumor testing should be carried out in individuals with CRC younger than 70 years, or those older than 70 years who fulfill any of the revised Bethesda guidelines (Table 1).
- If loss of MLH1/PMS2 protein expression is observed in the tumor, analysis of BRAF V600E mutation or analysis of methylation of the MLH1 promoter should be carried out first to rule out a sporadic case. If tumor is MMR deficient and somatic BRAF mutation is not detected or MLH1 promoter methylation is not identified, testing for germline mutations is indicated.
- If loss of any of the other proteins (MSH2, MSH6, PMS2) is observed, germline genetic testing should be carried out for the genes corresponding to the absent proteins (eg, MSH2, MSH6, EPCAM, PMS2, or MLH1).
ACG Clinical Guideline: Genetic Testing and Management of Hereditary Gastrointestinal Cancer Syndromes

Sapna Syngal, MD, MPH, FACG1,2,3, Randall E. Brand, MD, FACG4, James M. Church, MD, FACG5,6,7, Francis M. Giardiello, MD8, Heather L. Hampel, MS, CGC9 and Randall W. Burt, MD, FACG10

Am J Gastroenterol 2015; 110:223–262; doi: 10.1038/ajg.2014.435; published online 3 February 2015
LYNCH SYNDROME (LS)

Tumor testing and indications for genetic testing

**Summary statements**

1. All newly diagnosed colorectal cancers (CRCs) should be evaluated for mismatch repair deficiency.
2. Analysis may be done by immunohistochemical testing for the *MLH1/MSH2/MSH6/PMS2* proteins and/or testing for microsatellite instability (MSI). Tumors that demonstrate loss of *MLH1* should undergo BRAF testing or analysis for *MLH1* promoter hypermethylation.
3. Individuals who have a personal history of a tumor showing evidence of mismatch repair deficiency (and no demonstrated BRAF mutation or hypermethylation of *MLH1*), a known family mutation associated with LS, or a risk of ≥5% chance of LS based on risk prediction models should undergo genetic evaluation for LS.
Importance of evaluating mismatch repair deficiency outside the context of Lynch syndrome screening

Poor Survival Associated with the BRAF V600E Mutation in Microsatellite-Stable Colon Cancers

Wade S. Samowitz, Carol Sweeney, Jennifer Herrick, Hans Albertsen, Theodore R. Levin, Maureen A. Murtough, Roger K. Wolff, and Martha L. Slattery

1Department of Pathology, University of Utah Health Sciences Center; 2Department of Family and Preventive Medicine, Health Research Center, Salt Lake City, Utah and 3Kaiser Permanente Medical Center, Walnut Creek, California

BRAFV600E immunohistochemistry in conjunction with mismatch repair status predicts survival in patients with colorectal cancer

Christopher W Toon, Angela Chou, Keshani DeSilva, Joseph Chan, Jillian Patterson, Adele Clarkson, Loretta Sison, Lucy Jankova, and Anthony J Gill

1Department of Cancer Diagnosis and Pathology, Kolling Institute of Medical Research, St Leonards, NSW, Australia; 2Histopathology, North Ryde, NSW, Australia; 3Sydney Medical School, University of Sydney NSW, Australia; 4Department of Anatomical Pathology, SYDPATH, St Vincents Hospital, Darlinghurst, NSW, Australia; 5Department of Anatomical Pathology, Royal North Shore Hospital, Sydney, NSW, Australia; 6Bill Walsh Cancer Research, Kolling Institute of Medical Research, St Leonards, NSW, Australia and 7Kolling Institute of Medical Research, St Leonards, NSW, Australia
## Prevalence

<table>
<thead>
<tr>
<th></th>
<th>N=1426</th>
<th>BRAF wild-type</th>
<th>BRAFV600E mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proficient MMR</td>
<td></td>
<td>1057 (74.1%)</td>
<td>91 (6.4%)</td>
</tr>
<tr>
<td>Deficient MMR</td>
<td></td>
<td>94 (6.6%)</td>
<td>184 (12.9%)</td>
</tr>
</tbody>
</table>

## Prognosis

<table>
<thead>
<tr>
<th></th>
<th>BRAF wild-type</th>
<th>BRAFV600E mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proficient MMR</td>
<td>Intermediate</td>
<td>Poor</td>
</tr>
<tr>
<td>Deficient MMR</td>
<td>Good</td>
<td>Good</td>
</tr>
</tbody>
</table>
Clinical significance of molecular subgroup

<table>
<thead>
<tr>
<th>MMR proficient</th>
<th>BRAF WT</th>
<th>BRAFV600E mutant</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>○ 5FU monotherapy ○ anti-EGFR X PD1 inhibitor</td>
<td>○ 5FU monotherapy X anti-EGFR X PD1 inhibitor 5FU+Oxaliplatin+Irinotecan (Stage 4)</td>
</tr>
<tr>
<td>MMR deficient</td>
<td>X 5FU monotherapy ○ anti-EGFR ○ PD1 inhibitor</td>
<td>X 5FU monotherapy X anti-EGFR ○ PD1 inhibitor</td>
</tr>
</tbody>
</table>
Conclusion

• Universal screening for colorectal cancer is likely to become the future national standard of care

• *But this standard requires development of sufficient local and community infrastructure to appropriately handle genetic results before implementation*

Consensus Statement by the US Multi-Society Task Force on Colorectal Cancer
Gastroenterology 2014;147:502-526
Proposed AGPS Consensus Guidelines for Lynch syndrome screening

Consensus statements

- Mismatch repair immunohistochemistry is a phenotype rather than a genotype test. Therefore genetic counselling is not required before mismatch repair immunohistochemistry, microsatellite instability, BRAF mutation testing or hypermethylation testing is performed.
Proposed AGPS Consensus Guidelines for Lynch syndrome screening

Consensus statements

- All newly diagnosed colorectal cancers should be tested for mismatch repair deficiency by immunohistochemistry for mismatch repair proteins and/or microsatellite instability analysis. This can be performed on either the biopsy or resection specimen.

The value of evaluating mismatch repair deficiency is acknowledged not only for Lynch syndrome screening, but also as a prognostic factor and a predictive factor for chemotherapy.
Proposed AGPS Consensus Guidelines for Lynch syndrome screening

Consensus statements

- Ideally all colorectal cancers with abnormal MLH1 protein expression should undergo BRAFV600E mutation analysis as a surrogate marker of hypermethylation and MLH1 promoter methylation analysis if BRAF is wild type. Depending on the environment and available resources, a triage decision may need to be made.

If the tumour is BRAF wild type and negative for MLH1 promoter methylation, germline mutation analysis is indicated.
Proposed AGPS Consensus Guidelines for Lynch syndrome screening

Consensus statements

- Colorectal cancers with abnormal mismatch repair protein expression that does not involve MLH1 should undergo germline mutation analysis
Thank you