Explaining an Atypical IHC Pattern Using Paired Tumor/Germline Testing for Lynch syndrome

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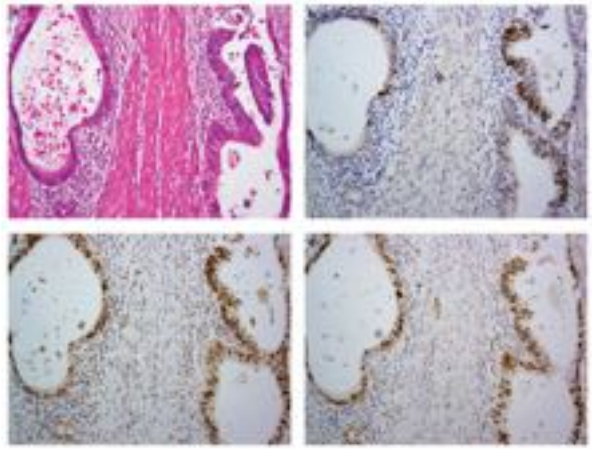
Conflict of Interest Disclosure

- I am a paid employee of Ambry Genetics.

Background

Seeking to explain cases with abnormal* MLH1 and/or PMS2 and MSH6 IHC staining



- Atypical but not uncommon IHC pattern
- Previously reported in 2% of CRC tumors[1]
- Abnormal MSH6 staining associated with somatic *MSH6* coding microsatellite (CM) mutations in some cases with this IHC pattern[1]

*Abnormal= absent/equivocal/weak staining

1) Shia J, et al. Secondary mutation in a coding mononucleotide tract in MSH6 causes loss of immunoexpression of MSH6 in colorectal carcinomas with MLH1/PMS2 deficiency. *Mod Pathol.* 2013; 26(1): 131-8.

Methods

- 30/1190 (2.5%) paired tumor/germline tests analyzed
  - Previous IHC results showed abnormal MLH1 and/or PMS2 & abnormal MSH6

- Germline and somatic findings assessed to determine whether paired tumor/germline testing results explained atypical IHC pattern

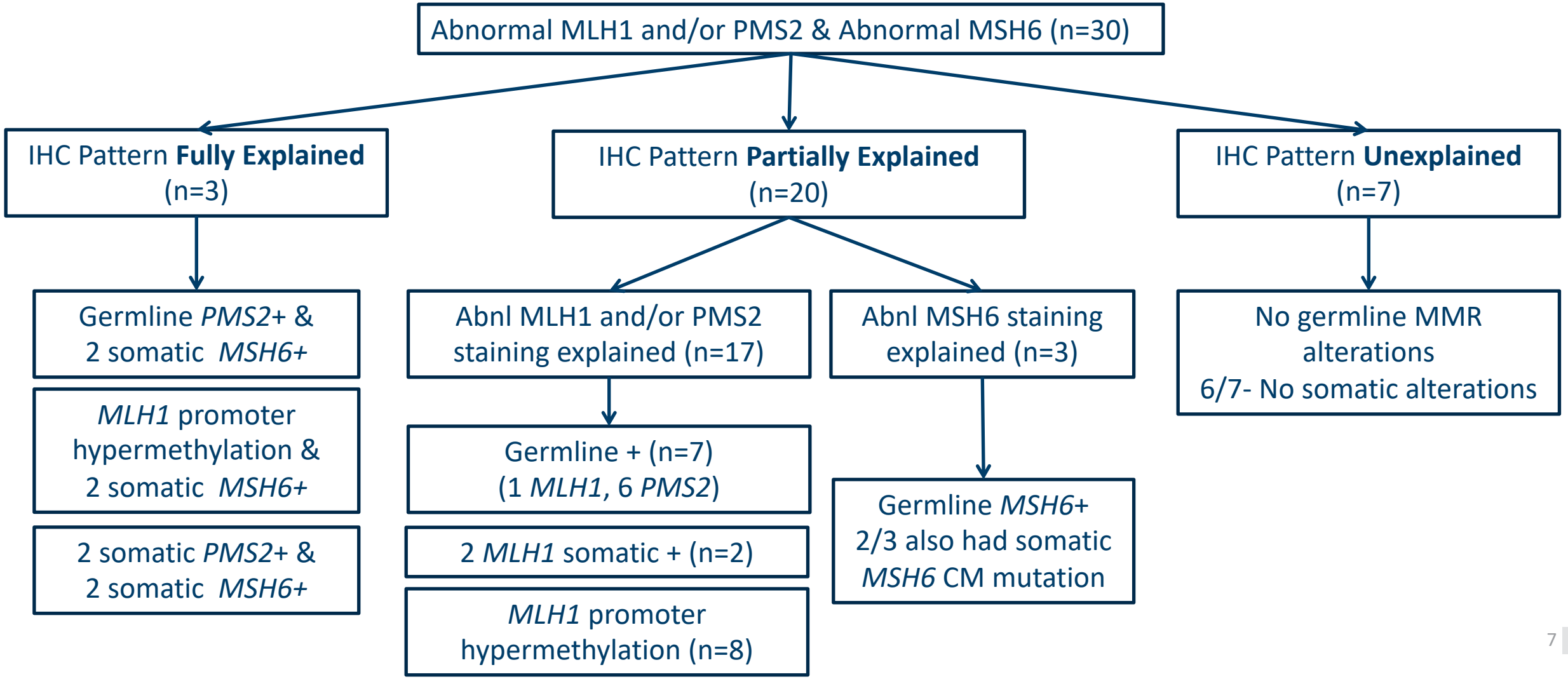
Explanation of Results

- Abnormal IHC staining of a protein considered “explained” by any of the following events in the corresponding gene:
  - Germline pathogenic mutation (+) or likely pathogenic variant (VLP)
  - Double somatic +/VLPs
  - Somatic +/VLP in addition to copy-neutral loss of heterozygosity (CN-LOH)
  - Promoter hypermethylation (*MLH1* only)

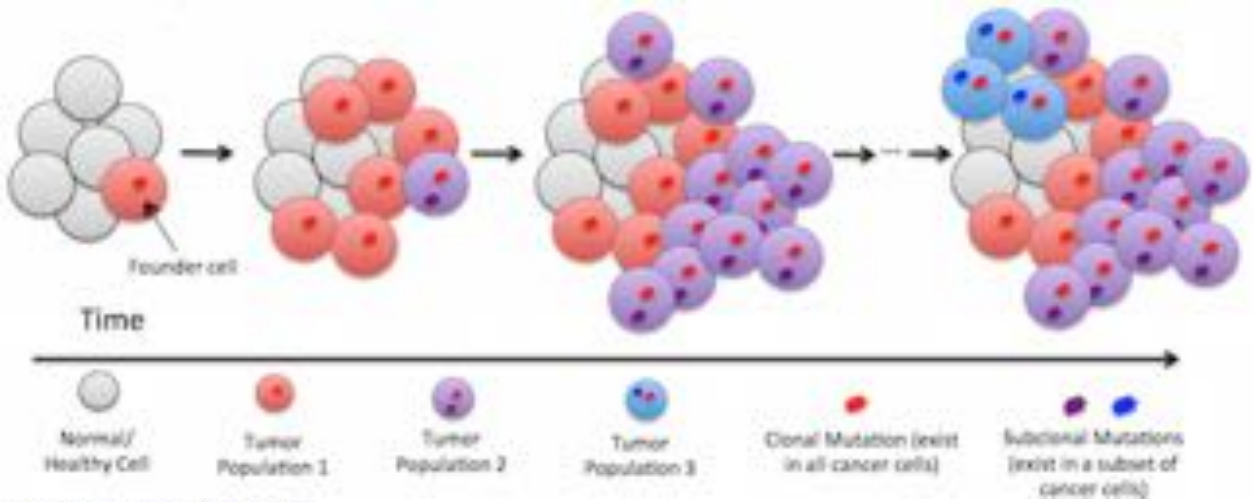
Explanation of Results

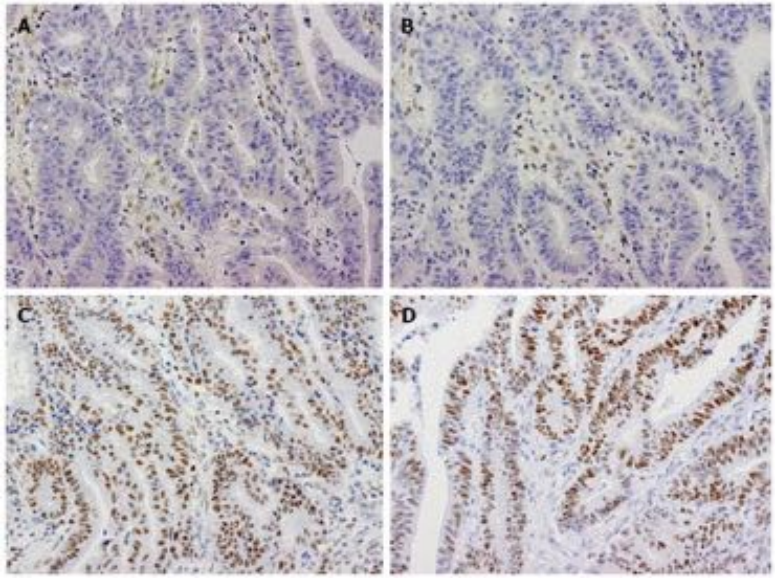
- “Fully Explained”
  - Abnormal MLH1 and/or PMS2 staining AND abnormal MSH6 staining explained by germline and/or somatic results
- “Partially Explained”
  - EITHER abnormal MLH1 and/or PMS2 staining OR abnormal MSH6 staining explained by germline and/or somatic results
- “Unexplained”
  - Paired testing results did not explain abnormal staining of MLH1, PMS2, or MSH6

Atypical IHC Cases



Discordant Results





Results- Fully Explained Cases

IHC result (MLH1/MSH6/PMS2)	Germline findings	Somatic findings[a]
Present/**Equivocal/Absent**	**PMS2+**	**MSH6+ x2 (c.3261delC, c.741dupA)**
Absent/Absent/Absent	None	**MPH[b], MLH1+, MSH6+ x3 (c.3261delC),** MSH6 VUS
Present/**Absent/Absent**	None	**PMS2+ x2, MSH6+, MSH6 VLP**, PMS2 VUS, MSH6 VUS

[a] Alterations in parentheses show *MSH6* coding microsatellite (CM) mutations
[b] MPH=*MLH1* promoter hypermethylation

Results- Partially Explained; Concordant Cases

- Paired testing results were concordant with atypical IHC in 11/30 cases (37%)
  - Did not explain abnormal MSH6 staining due to single somatic CM mutation
  - 9 colon, 2 endometrial tumors
  - 5 germline *PMS2*+
  - 5 *MLH1* promoter hypermethylation
  - 1 somatic *MLH1*+ & CN-LOH
  - *MSH6* CM mutations: 10 exon 5 C8 (c.3261delC/c.3261dupC); 1 exon 5 T7 (c.3312delT)

Results- Partially Explained; Concordant Cases

- Results similar to previously published data from Shia et al (2013)
  - Included 5 CRC tumors with absent MLH1 and/or PMS2 and “scanty” (<5%) MSH6 staining on IHC
  - 4/5 cases had somatic *MSH6* CM mutations
    - Only looked at exon 5 C8

Conclusions

Paired tumor/germline testing in atypical IHC cases

- Paired tumor/germline testing fully explained IHC in 10% of cases
  - Germline testing alone would not have fully explained these cases
- Somatic *MSH6* CM mutations contribute to abnormal MSH6 IHC staining
  - Supports previously published data
- Including somatic MMR gene analysis increases the likelihood of complete IHC explanation in atypical cases

Questions?