Multiple Mutations in Lynch syndrome: Case Report and Experience of One Clinical Laboratory

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BACKGROUND

- Lynch syndrome is the most common cause of hereditary colon cancer, and is typically caused by a single mutation in one of the mismatch repair (MMR) genes: *MLH1, MSH2, MSH6* or *PMS2*, or by a gross deletion in *EPCAM*.
- Carrying two MMR mutations in the same gene in *trans* is associated with a diagnosis of constitutional mismatch repair-deficiency syndrome (CMMR-D)
- CMMR-D is characterized by colonic polyposis; hematologic, brain, and gastrointestinal malignancies; and neurofibromas, all of which typically present in childhood
- Limited data exist on the prevalence of individuals who have two MMR mutations in the absence of a CMMR-D phenotype.

Table 1: Lynch Syndrome Multiple Mutation Experience at Ambry

Gene	Mutations	Phase	History
MSH2	c.942+3A>T, c.2785C>T	In *cis*	Endometrial ca-42; fam hx of colon
MSH2	c.1915C>T, c.2211-1G>T	No phase info	Sebaceous adenoma, 51; adopted
MSH6	c.3G>T, c.3725G>A	Likely *trans*	Colon ca x3-23, 6-9 polyps; mother 9 adenomas
MSH6	c.2147_2150del, c.3725G>A	No phase info	Colon ca-16, fam hx not provided
PMS2	c.1831dupA, EX10del	In *trans*	ALL-10, 6 CAL's; fam hx of polyps
PMS2	c.736_741del6ins11, c.1831dupA	Likely *trans*	6-9 adenomas-14, NHL-3, 4 CAL's
PMS2	c.2500_2501delATinsG, c.2T>A	Likely *trans*	Glio-8, CAL's; fam hx of colon on both sides
PMS2	c.1A>T, c.746_753del8	No phase info	100+ adenomas-24, PNET-19, RB-2, lymph-22, brother ALL-8
MLH1	EX15del (homozygous)	In *trans*	RB-1, CAL's; fam hx of colon on both sides, consanguinity
MLH1	c.2218dupA (homozygous)	In *trans*	Lymphoma-5, fam hx of colon on both sides, consanguinity
MSH2	c.2152C>T (homozygous)	In *trans*	Brother "bone marrow ca"- 4, fam hx stomach ca
MSH6	c.10C>T (homozygous)	In *trans*	20-99 adenomas-17, mat. g-mother panc, pat. cousin brain-15
MSH6	c.3701_3706dupAACTTG (homo)	In *trans*	Glioma-12, 6+ CAL's; fam hx uterine and stomach, consanguinity
PMS2	c.1A>G (homozygous)	In *trans*	Rectal ca-20, no fam hx provided
PMS2	c.2458dupA (homozygous)	In *trans*	2 sibs with unspecified ca <18; consanguinity
PMS2	c.1376C>G (homozygous)	In *trans*	Colon ca-20, 6-9 polyps, ALL-3; consanguinity

METHODS

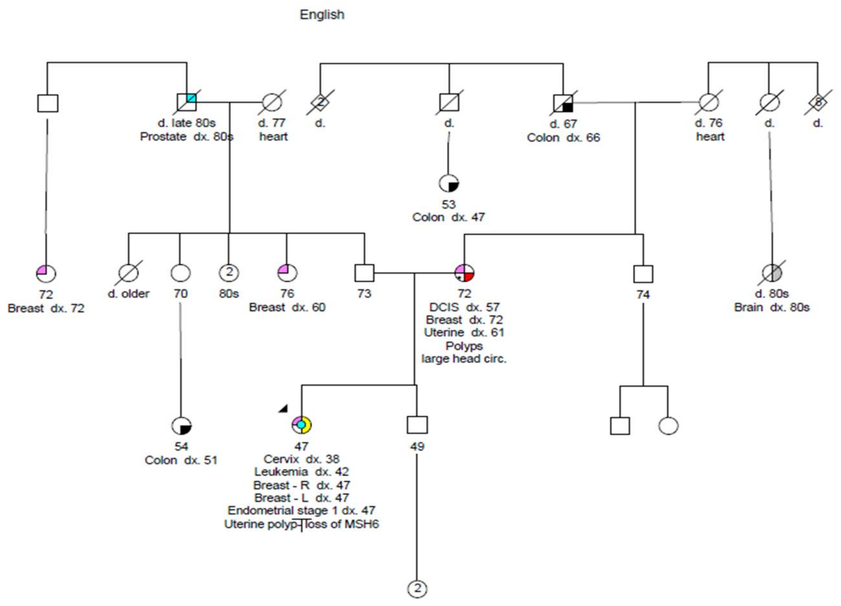
- To explore the prevalence and phenotype of patients with 2 MMR mutations, data was collected on 29,907 individuals sequenced for 2 or more MMR genes at Ambry.
- Patients underwent multi-gene panel or targeted Lynch syndrome testing.
- Clinical histories were reviewed to assess the phenotypes of individuals carrying multiple MMR mutations.

RESULTS

- Probands from 16 families were found to carry two mutations in the same MMR gene (Table 1):
  - 1 proband had mutations confirmed in *cis*
  - 12 probands with CMMR-D phenotype
  - 3 probands with unknown phase

Case Report: Clinical History and Genetic Testing

Pedigree



Personal History

- 47 year old women presented with 5 cancer primaries:
  - Adenocarcinoma *in situ* of the cervix (age 38)
  - Chronic lymphocytic leukemia (age 44)
  - Bilateral invasive ductal carcinoma (age 47)
  - Endometrial adenocarcinoma (age 47)
- Immunohistochemical analysis of endometrial adenocarcinoma revealed loss of *MSH6* protein expression

Maternal Family History

- Mother diagnosed with bilateral breast cancer at ages 57 and 72, and uterine cancer at age 61
- Grandmother and second cousin diagnosed with colon cancer at ages 66 and 47, respectively

Paternal Family History

- Aunt diagnosed with breast cancer at age 60
- 1st cousin diagnosed with colon cancer at age 51
- Grandfather diagnosed with prostate cancer in his 80's

Genetic Testing Results

- Proband underwent Ambry's CancerNext panel on a cultured fibroblast sample (due to history of CLL), which revealed:
  - *MSH6* pathogenic mutation: c.3939_3957dup19
  - *PMS2* pathogenic mutation: c.736_741del6ins11
  - *MUTYH* pathogenic mutation: p.G396D
  - *APC* variant of uncertain significance: p.R2166Q
- Proband's mother underwent Ambry's CancerNext panel, which revealed:
  - *MSH6* pathogenic mutation: c.3939_3957dup19
  - *PMS2* pathogenic mutation: c.736_741del6ins11
  - *APC* variant of uncertain significance: p.R2166Q

Case Report: Interpretation

- Patient and her mother have Lynch syndrome
  - Likely explains proband and her mother's endometrial cancer, and possibly the breast cancer history
  - Unclear if Lynch syndrome is contributing to proband's leukemia and cervical cancer history
- Carrier for *MUTYH*
  - Increased risk for colon cancer, adenomas, and breast cancer (~2 fold increase)
  - May contribute to proband's breast cancer history
- *APC* variant of uncertain significance
  - Rare and limited evidence
  - Phenotype doesn't match FAP

Case Report: Mutation Details

***MSH6* c.3939_3957dup19**

- Frameshift with predicted alternate stop codon (p.A1320Sfx*5)
- Several reports in literature in families concerning for Lynch:
  - Chong G et al. *Hum Mutat*. 2009;30(8):E797-812
  - Goodfellow PJ et al. *Proc Natl Acad Sci USA*. 2003;100(10):5908-5913
  - Hampel H et al. *J Clin Oncol*. 2008;26(35):5783-5788

***PMS2* c.736_741del6ins11**

- Frameshift with predicted alternate stop codon
- Multiple studies identified mutation in Lynch syndrome families: Clendenning, M et al. *Hum Mutat*. 2006 Nov;27(11):1155; Clendenning, M et al. *J Med Genet*. 2008 Jun;45(6):340-5; Talseth-Palmer BA et al. *Hered Cancer Clin Pract*. 2010 May 21;8(1):5; Herkert, J et al. *Eur. J. Cancer* 2011 May;47(7):965-82; Buchanan, D et al. *J Clin Oncol*. 2014 Jan 10;32(2):90-100
- Family studies determined this is a founder mutation enriched in British and Swedish individuals, may be reduced penetrance: Clendenning, M et al. *J Med Genet*. 2008 Jun;45(6):340-5

TAKE-HOME POINTS

- In a cohort of nearly 30,000 cases tested for two or more MMR genes, we identified 16 families with mutations in the same MMR gene
- We identified a single family with mutations in two different genes: *MSH6* and *PMS2*
- For the families with two mutations in the same gene: most were suggestive of CMMR-D
- While further studies are needed, this single report suggests that digenic mutations in MMR genes are not associated with a classic CMMR-D phenotype
- These data also suggest that the presence of two MMR gene mutations is a rare event, and occurs more often in the same gene than in two different genes