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RNA Genetic Testing Provides a Clear Diagnosis

CASE STUDY

Clinical Scenario

WHO IS THE PATIENT?

- 55 year old female
- Endometrial cancer at 54y; immunohistochemistry - absent MSH6
- Breast cancer at 54y

WHAT IS THE FAMILY HISTORY?

PATERNAL FAMILY HISTORY

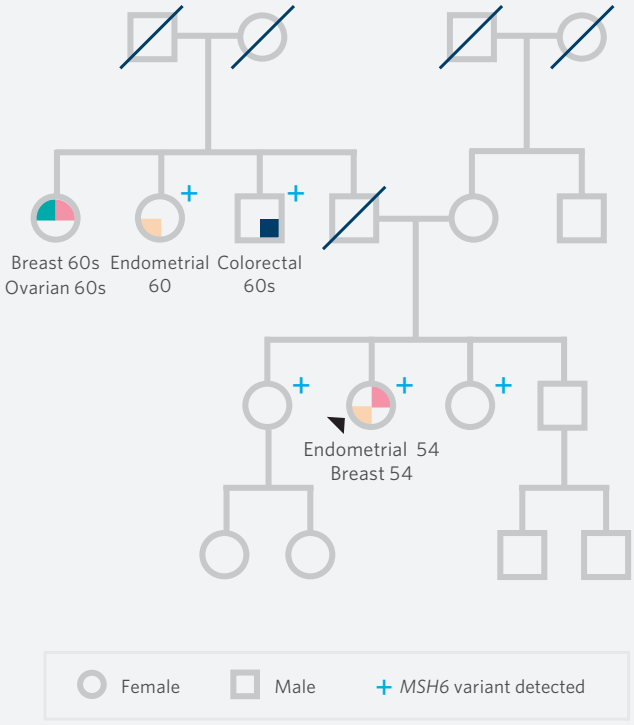
- Aunt with endometrial cancer at 60y
- Uncle with colorectal cancer in 60s
- Aunt with ovarian and breast in 60s

MATERNAL FAMILY HISTORY

- No history of cancer

GENETIC TESTING CRITERIA

- Patient meets NCCN® genetic testing criteria for Lynch syndrome as well as *BRCA1* and *BRCA2*[1,2]



Genetic Testing

Provider ordered **Ambry's CancerNext-*Expanded***

GENETIC TEST RESULTS

- Variant of Unknown Significance (VUS) identified in *MSH6* (c.3802-7_3802-4delTCTT)
- Same VUS was identified in multiple family members (see pedigree above)

Using RNA Genetic Testing to Provide Clarity

RNA GENETIC TESTING

RNA analysis was completed for both sisters with the *MSH6* variant

RNA RESULTS

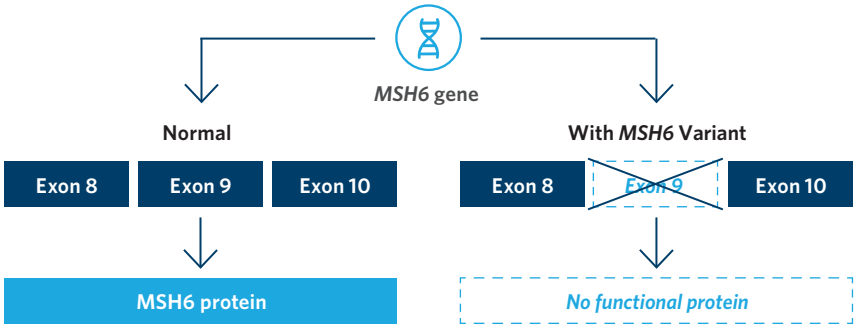
RNA analysis revealed that the variant results in skipping of Exon 9

RESULT INTERPRETATION

Variant is now considered likely pathogenic (disease-causing), which is consistent with a diagnosis of Lynch syndrome

MSH6 Variant Results in Skipping of Exon 9

This segment of DNA is used when coding for the MSH6 protein. Therefore, this variant impacts the function of the *MSH6* gene, providing evidence to classify it as a variant, likely pathogenic (VLP).



RNA Genetic Testing Clarified a Diagnosis of Lynch Syndrome

CLARIFIED A DIAGNOSIS

- Patient and family members with the identified *MSH6* variant now have a clear diagnosis of Lynch syndrome
- Additional paternal family members can be tested to determine their cancer risks

INFORMED INCREASED LIFETIME CANCER RISKS

- Colorectal
- Uterine
- Ovarian
- Other

ENABLED PERSONALIZED MEDICAL MANAGEMENT

- Medical management per Lynch syndrome guidelines[1] is now indicated including:
  - Colonoscopies every 1-2 years
  - Consider total abdominal hysterectomy/ bilateral salpingo-oophorectomy
  - Possible upper endoscopy every 3-5 years

Points For Your Practice

- This patient and several family members were initially found to have a variant of unknown significance in *MSH6*.
- The addition of RNA studies enabled us to clarify the significance of the *MSH6* variant and provide a clinically actionable diagnosis of Lynch syndrome for this family.
- Combining RNA genetic testing with DNA testing decreases variants of unknown significance and increases actionable results for patients.

REFERENCES

1. National Comprehensive Cancer Network®. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Genetic/Familial High-Risk Assessment: *Colorectal*. Version 2.2016
2. National Comprehensive Cancer Network®. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Genetic/Familial High-Risk Assessment: *Breast and Ovarian*. Version 2.2019.

Based on information from an actual patient case; individual identifiers have been modified to protect patient privacy.

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RNA Genetic Testing Clarified a Negative Result

CASE STUDY

Clinical Scenario

WHO IS THE PATIENT?

- 50 year old female
- No personal history of cancer

WHAT IS THE FAMILY HISTORY?

PATERNAL FAMILY HISTORY

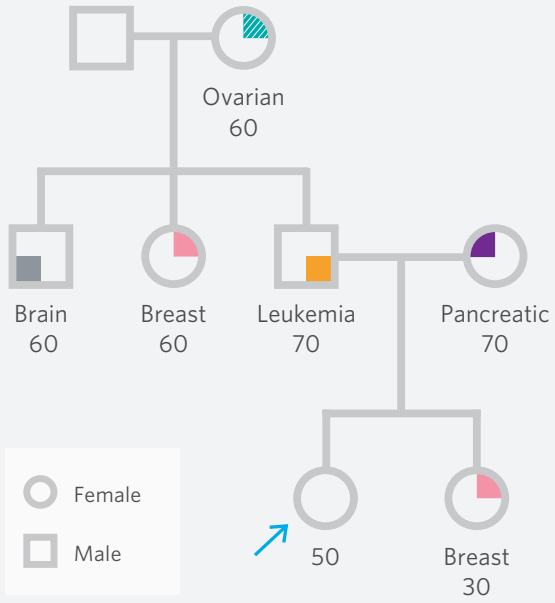
- Father with leukemia at 70y
- Aunt with breast cancer at 60y
- Grandmother with ovarian cancer at 70y
- Uncle with brain cancer at 60y

MATERNAL FAMILY HISTORY

- Mother with pancreatic cancer at 70y

GENETIC TESTING CRITERIA

- Patient meets NCCN® genetic testing criteria for *BRCA1* and *BRCA2*



Genetic Testing

Provider ordered **Ambry's OvaNext® +RNAinsight™**

GENETIC TEST RESULTS

- Variant identified in *MSH2* (c.1077-3C>T)
- Final classification was "variant, likely benign" (VLB) and patient received a negative report

Using RNA Genetic Testing to Provide Clarity

RNA GENETIC TESTING

Simultaneous RNA genetic testing was completed

RNA RESULTS

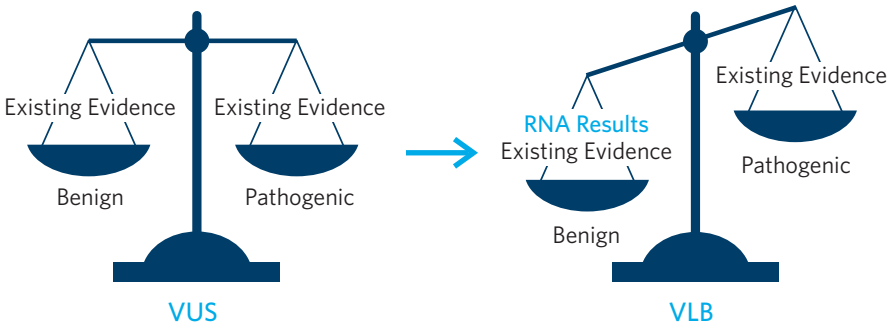
RNA genetic testing did not detect any significant abnormal transcripts in *MSH2*

RESULT INTERPRETATION

Variant was classified as VLB and the patient received a negative report

RNA Evidence Supports Likely Benign Classification

Using the American College of Medical Genetics criteria[2], the evidence available prior to RNA testing was suggestive of a benign classification, however, the confirmation of "no abnormal splicing" enabled us to change the result from variant of unknown significance (VUS) to variant, likely benign (VLB).



RNA Genetic Testing Enabled a Negative Result

PROVIDED CLEARER RESULTS

- Patient received a negative test report from initial testing
- Avoided a VUS on final report

AVOIDED ADDITIONAL TESTING

- Family studies to clarify the significance of the variant are not needed

CLARIFIED NEXT STEPS

- Cancer risk(s) and management recommendations based on personal and family history
- Additional genetic testing may be considered for family members

Points For Your Practice

- This patient was identified to have a variant in *MSH2* that would have been classified as VUS based on existing evidence.
- The addition of RNA evidence enabled us to classify the *MSH2* variant as a VLB and provide a negative report to the patient avoiding unnecessary confusion and uncertainty.
- Combining RNA genetic testing with DNA testing decreased variants of unknown significance and provided clearer results for patients.

REFERENCES

1. National Comprehensive Cancer Network®. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Genetic/Familial High-Risk Assessment: *Breast and Ovarian.* Version 2.2019.
2. Richards S. *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24

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RNA Genetic Testing Helped Identify a New Mutation Missed by DNA Alone

CASE STUDY

Clinical Scenario

WHO IS THE PATIENT?

- 43 year old female
- History of >10 colorectal polyps first detected at 28y
- No personal history of cancer

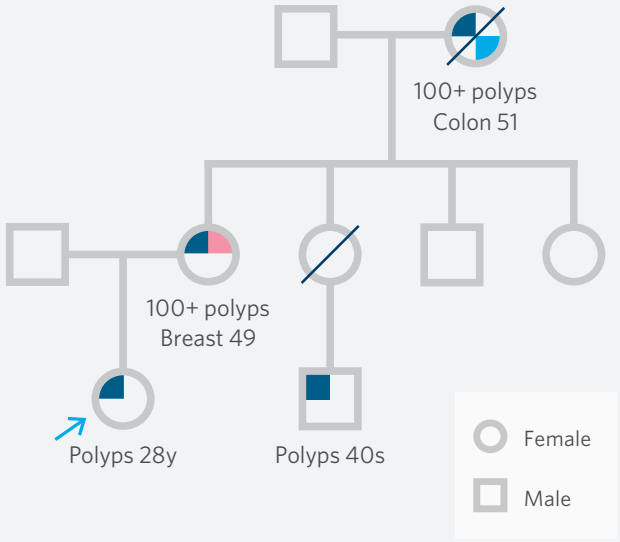
WHAT IS THE FAMILY HISTORY?

PATERNAL FAMILY HISTORY

- No paternal history of cancer

MATERNAL FAMILY HISTORY

- Mother with 100+ polyps and breast cancer at 49y
- Grandmother with 100+ polyps and colorectal cancer at 51y
- First cousin with colorectal polyps in his 40s



Genetic Testing

Provider ordered **Ambry's CancerNext®**

GENETIC TEST RESULTS

- Variant outside of reporting range identified in *APC* (c.423-11A<G)
- Reported after RNA genetic testing as "variant, likely pathogenic" (VLP) and patient received a diagnosis of familial adenomatous polyposis (FAP)
- Concurrent RNA testing prevented a clinical false negative

RNA Genetic Testing Results Demonstrated Abnormal Splicing

RNA GENETIC TESTING

Completed RNA genetic testing

RNA RESULTS

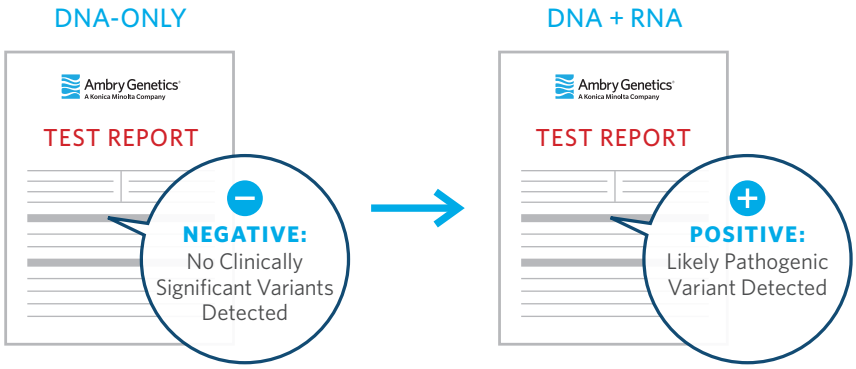
RNA genetic testing results demonstrated that this variant results in abnormal splicing

RESULT INTERPRETATION

Variant was classified and reported as VLP and results consistent with a diagnosis of FAP

RNA Evidence Supports Likely Pathogenic Classification

With standard DNA-only testing, this variant may have been missed because it is located outside of the reporting range. RNA evidence demonstrated that this variant results in abnormal splicing[1], which combined with other existing evidence supports a VLP classification[2].



RNA Genetic Testing Clarified a Diagnosis of FAP

CLARIFIED A DIAGNOSIS

- Patient received clear diagnosis of FAP, which is consistent with her personal and family history
- Additional family members can be tested to determine their cancer risks

INFORMED INCREASED CANCER RISKS

- Colorectal
- Small bowel
- Stomach
- Pancreatic
- Other

ENABLED PERSONALIZED MEDICAL MANAGEMENT[3]

- Annual colonoscopy beginning at 10-15y
- Colectomy (age individualized by polyp burden)
- Upper endoscopy starting at 20-25y
- Annual thyroid exam
- Annual physical exam

Points For Your Practice

- This patient was identified to have an *APC* variant outside of the reporting range.
- RNA evidence enabled accurate classification of the *APC* variant as a VLP and provided a clear diagnosis of FAP in real time.
- Combining RNA genetic testing with DNA testing expands the reporting range for clinically actionable mutations.

REFERENCES

1. Ambry Genetics, internal data on file.
2. Richards S. *et al.* Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015 May;17(5):405-24
3. National Comprehensive Cancer Network®. NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®). Genetic/Familial High-Risk Assessment: Colorectal. Version 1.2019

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