Case of a synonymous variant in *MLH1* with clinical significance

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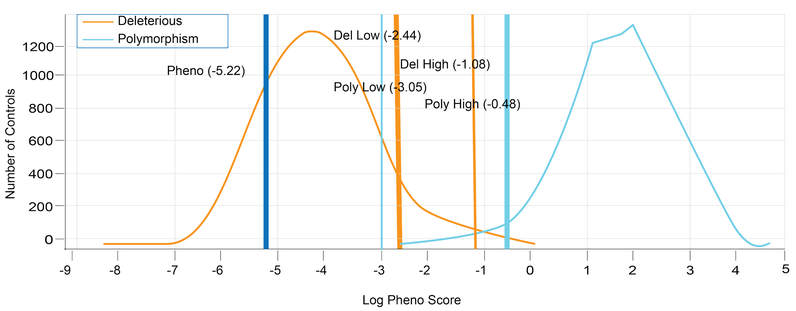
BACKGROUND

- Variant classification is based on the evidence available at the time of classification, and classifications can change as the science evolves and as more evidence becomes available.
- Although rare, occasionally new evidence leads to the reclassification of variants previously classified as benign, or pathogenic, which can greatly impact management recommendations.
- Here we report a case study involving the unusual reclassification of a variant initially classified as benign.

VARIANT CLASSIFICATION

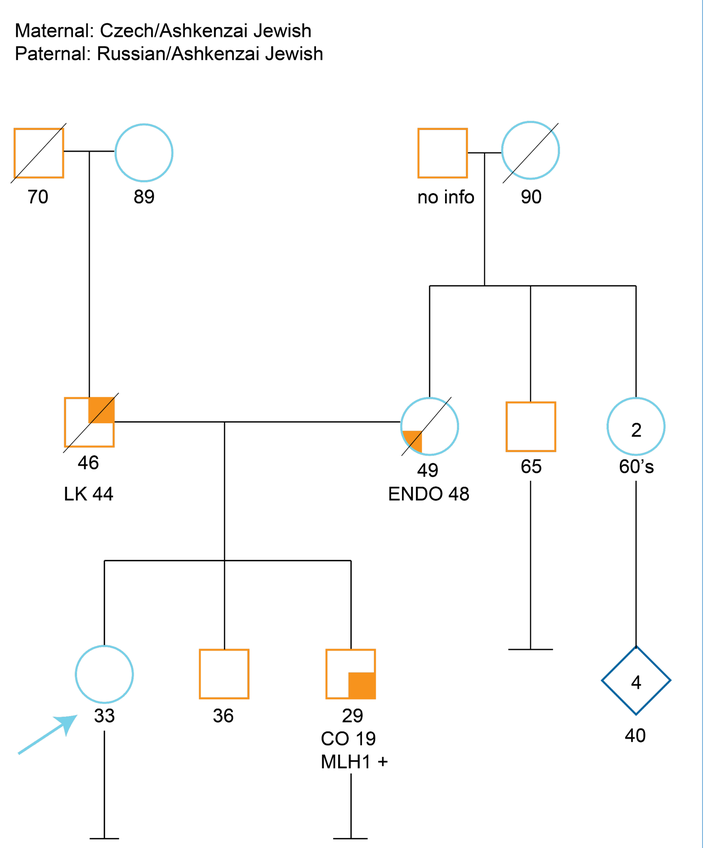
- *MLH1* c.27G>A (p.Arg9Arg) is a synonymous variant in the middle of the exon not expected to impact splicing initially classified as benign.
- Subsequent to the initial benign classification, Ward et al. indicated this variant correlated with reduced transcriptional activity[1] leading to a re-evaluation of the variant and the reclassification to uncertain.
- Although this variant is not predicted to impact splicing, RNA studies were done, as this is a silent variant, and the mechanism of impact was unknown. RNA splicing studies did not detect any aberrant splicing.
- Continued analysis of the variant with the laboratory's history weighting algorithm indicates that this variant is associated with more severe personal and family histories of cancer, consistent with pathogenic variants in *MLH1* (Fig. 1).[2]
- Although the exact mechanism of impact is unclear, the collective evidence indicates this variant is associated with increased cancer risks and Lynch syndrome.

Figure 1. Pheno for Mutation ID 9989652



CASE REPORT

Figure 2. Family History



- The proband, diagnosed with colon cancer at age 19, originally underwent testing for Lynch syndrome in 2010.
- Test results were reported as negative, given that the c.27G>A (p.Arg9Arg) *MLH1* variant was classified at the time as benign.
- A few years later, the proband was issued an amended report, revealing the updated classification to variant of uncertain significance (VUS).
- In 2018, the variant was again reclassified, this time to likely pathogenic.
- Subsequently, the proband's sister underwent cascade testing, identifying the c.27G>A *MLH1* variant.

Figure 3. Test Results and Interpretation

Specimen

Specimen Type:	**Blood**
Draw Date:	**March 16, 2010**
Accession Date:	**March 17, 2010**
First Report Date:	**March 25, 2010**
Report Date:	**July 6, 2018**

Test Results and Interpretation

Genetic Variant, Suspected Deleterious

Test Performed:	Result:	Interpretation:
MLH1 Sequencing	R9R (27G>A)	**Suspected Deleterious**
Comprehensive Rearrangement	No Mutation Detected	No Mutation Detected
MSH2 Sequencing	No Mutation Detected	No Mutation Detected
Comprehensive Rearrangement	No Mutation Detected	No Mutation Detected
MSH6 Sequencing	No Mutation Detected	No Mutation Detected

CONCLUSIONS

This case highlights the evolving nature of variant classification as well as the importance of laboratories evaluating new evidence for variants of all classifications. In addition, this case illustrates the importance of healthcare providers following up with patients with updates to their genetic test results.

FUTURE STEPS

We plan to perform germline *MLH1* promoter analysis to assess the hypothesis that the pathogenicity is due to induction of hypermethylation.

REFERENCES

1. Ward RL, Dobbins T, Lindor NM, et al. Identification of constitutional MLH1 epimutations and promoter variants in colorectal cancer patients from the Colon Cancer Family Registry. Genet Med. 2013;15:25–35

2. Pruss D, Morris B, Hughes E, Eggington JM, Esterling L, Robinson BS, van Kan A, Fernandes PH, Roa BB, Gutin A, Wenstrup RJ, Bowles KR Development and validation of a new algorithm for the reclassification of genetic variants identified in the *BRCA1* and *BRCA2* genes. Breast Cancer Res Treat. 2014 Aug;147(1):119-32.

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