Utilization of normal adjacent tissue for germline confirmation

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I. Introduction

Post mortem genetic testing is testing that is performed on a sample after an individual has died. The goal is often to try to identify the cause of death and/or identify if blood relatives are at-risk for a genetic disease.

Formalin-fixed, paraffine-embedded (FFPE) tumor tissue samples often contain a portion of normal unaffected tissue.

Utilization of this normal tissue within stored FFPE samples can provide germline samples for paired tumor/germline testing.

II. Objectives

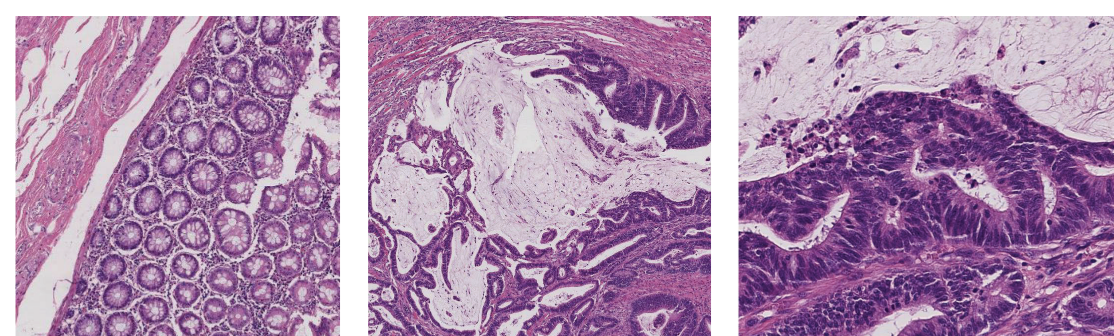
CASE PRESENTATION: In this discussion, we review a case from 1999 in which we were able to extract DNA from normal adjacent tissue (NAT) within the FFPE tumor sample slides and provide confirmation of an underlying MMR (*MSH2* del 2 -> 8) germline pathogenic variant consistent with Lynch Syndrome.

Immunohistochemistry performed on both of this patient's tumors samples showed loss of *MSH2* and *MSH6* expression, and intact nuclear expression of *PMS2*. No germline testing had been performed previously.

Mismatch repair (MMR) somatic tumor testing at Impact Genetics using next-generation sequencing (NGS) was performed on both tumor samples for *MSH2* and *MSH6* genes.

III. Methods

***MLH1/MSH2/MSH6/PMS2/EPCAM* Somatic Tumor MMR Sequencing and Deletion/Duplication**



Normal Mucosa and Muscularis — 20x tumor with Mucin — 100x tumor with Mucin

1. DNA extracted from FFPE slides
2. Sequence analysis of all coding exons and flanking intronic regions of up to 5 genes using NGS.

DNA corresponding to all *MLH1*, *MSH2*, *MSH6*, *PMS2* and *EPCAM* coding regions as well as 25 base pairs (bp) of non-coding flanking DNA was captured using DNA hybridization probes. Captured DNA was sequenced using Illumina sequencing technologies and processed using the Data-Driven Medicine (DDM) Bioinformatics pipeline (Sophia Genetics). Minimum NGS coverage is 1000X for all exons and ±25 bp of flanking intronic sequencing. All regions with coverage that does not meet this threshold are assessed by Sanger sequencing. All pathogenic, likely pathogenic and uncertain NGS variants are confirmed by Sanger sequencing.

3. Multiplex Ligation-dependent Probe Amplification (MLPA) is used to assess for large single or multi-exon deletions and duplications.

IV. Results

TUMOR TESTING

IHC/MSI: IHC = loss of nuclear expression of *MSH2* and *MSH6* (intact nuclear expression of *MLH1* & *PMS2*)

BRAF V600E – Negative

Detected in tumor	Detected in blood	Gene	Variant	Classification	Variant allele frequency
Yes	Yes	MSH2	del2 -> 8	Pathogenic	50%
Yes	No	MSH2	c.942+2_942+13delinsG	Likely Pathogenic	28%
Yes	No	MSH2	c.1697delA (p.Asn566Ilefs*24)	Pathogenic	26.6%

No reportable variants detected in *MLH1*, *MSH6*, *PMS2*, or *EPCAM* by NGS in tumor sample

D. 56
Rectal Ca
Cancer
Cervical Ca
Dx. 52
D. 50's
Bowel Ca
Bowel Ca dx. 37 & 55
Endometrial dx 46
No DNA available
D. 60's
Lung Ca
D. 40's
Bowel Ca
D. 55
Lung Ca
MSH2
Neg
Sebaceous
Adenoma
Ovarian CA
dx.49
No DNA
available
MSH2
Neg

INTERPRETATION:

Germline del 2 -> 8 variant and other somatic variants identified.

Del 2 -> 8 pathogenic variant: detected in both this patient's NAT and tumor; this deletion was confirmed using two independent MLPA kits (MRC Holland P003-D1 and P248-B1). A similar deletion has been described in the InSiGHT database (individual ID: 00023445, Genuardi et al., unpublished) **THIS CONFIRMS A GENETIC DIAGNOSIS OF LYNCH SYNDROME**.

OTHER VARIANT IDENTIFIED:

c.942+2_942+13delinsG likely pathogenic variant: (hemizygous) To our knowledge, this deletion/insertion has not previously been reported in either affected or control datasets, however, similar deletions have been reported in ClinVar (SCV000284197: c.942+2_942+6del) as a pathogenic germline variant, as well as somatically in COSMIC (COSM5829472: c.942+3_942+19del) in one large intestine cancer. This deletion/insertion is predicted by all splice prediction algorithms to disrupt the endogenous intron 5 donor splice site, leading to errors in splicing. As this patient carries a deletion of *MSH2* exon 2-8 on one allele explains the hemizygous presentation of this variant. Given the current information, we interpret this variant to be likely pathogenic.

c.1697delA(p.Asn566Ilefs*24 pathogenic variant: (heterozygous) This deletion has been described once in ClinVar (SCV000184301) as a germline pathogenic variant in a patient with Lynch syndrome. This deletion is predicted to result in the formation of a premature termination codon. Given the current information, we interpret this variant to be pathogenic.

V. Conclusions

This case illustrates the capability of confirming underlying Lynch syndrome by using DNA obtained from NAT present in FFPE tumor samples. This is a valuable tool when the proband is deceased and DNA obtained from blood is not available.

Molecular confirmation of the pathogenic germline variant allows for cascade testing of at risk family members for the familial *MSH2* variant.

Paired tumor/NAT testing can allow for reflex testing for informative deceased relatives from decades ago if enough DNA is present in FFPE samples.

VI. References

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VII. Acknowledgements

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