To NMD and Beyond: Identifying Mutations in the 3' Terminus of Mismatch Repair Genes

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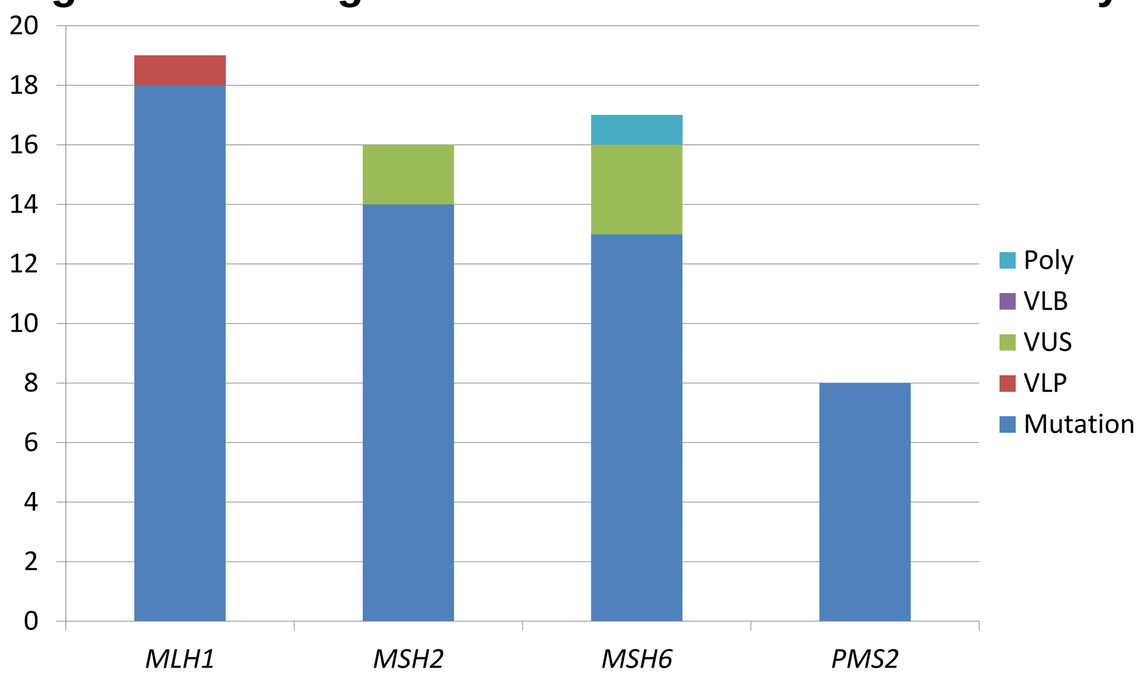
BACKGROUND

- Protein truncating alterations in tumor suppressor genes such as the mismatch repair genes *MLH1*, *MSH2*, *MSH6* and *PMS2* are typically deleterious[1]
- Variants that occur at the 3' terminus of genes are not expected to trigger nonsense-mediated decay (NMD), resulting in a truncated protein
- What functional impact the loss of these distal amino acids may cause remains unclear without additional evidence
- Our aim was to describe the most 3' protein truncating alterations classified as pathogenic or likely pathogenic that have been identified in the Ambry Genetics cohort

Fig 1. Truncating Alterations Distal of NMD Boundary



METHODS

- The NMD boundary is defined as being 55 nucleotides from the 3' end of the penultimate exon in each gene[2]
- We queried the Ambry Genetics variant database for all indels, frameshift and nonsense alterations identified to date downstream of the NMD boundary for *MLH1*, *MSH2*, *MSH6* and *PMS2*
- Publicly available databases were also searched for entries identified downstream of the most distal alteration found at Ambry

Figure 2. Most Distal Truncating MMR Mutations

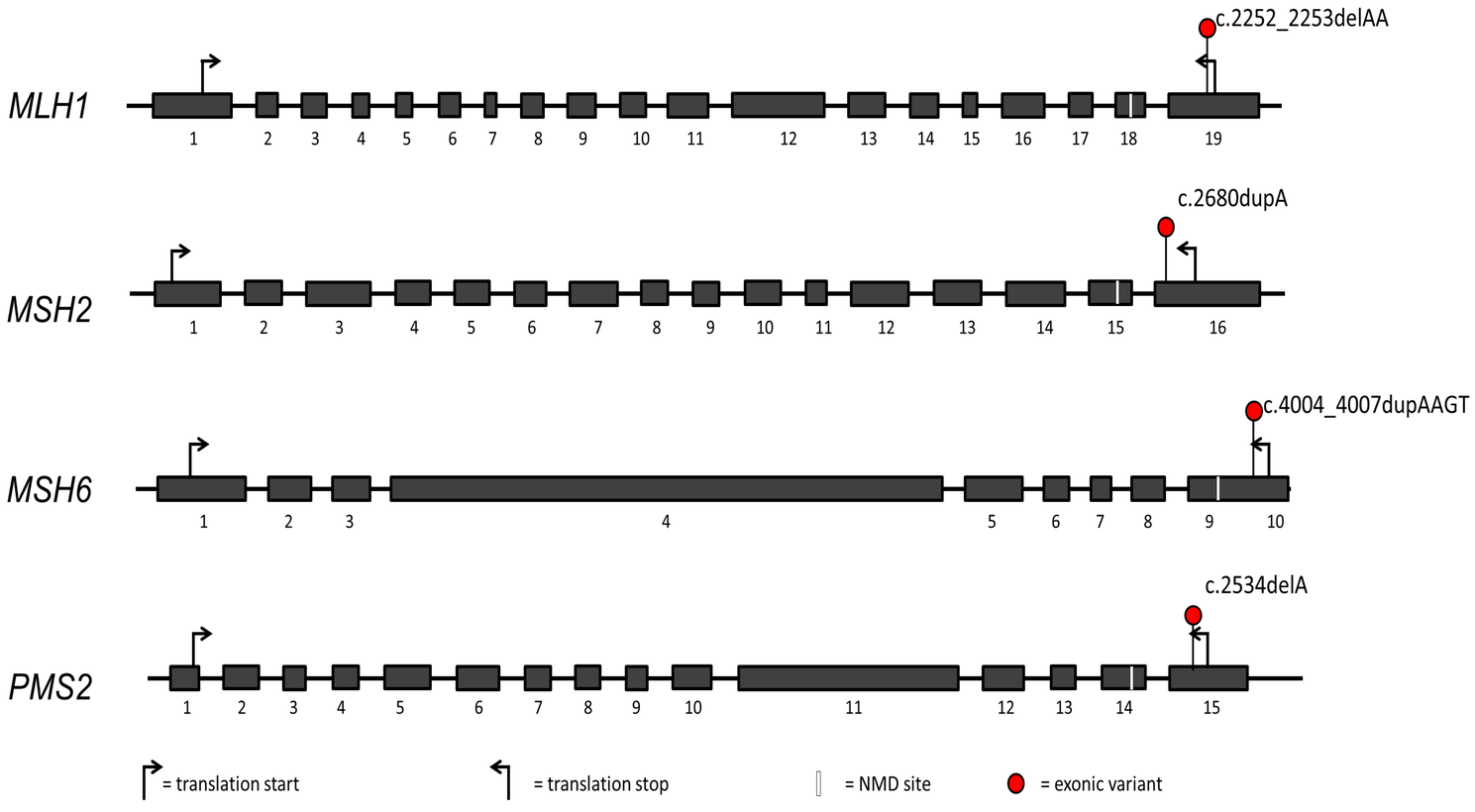


Table 1. Distal Mutation Evidence

Gene	Alteration	Classification	Key Evidence
MLH1	c.2252_2253delAA (p.K751Sfs*3)	Pathogenic Mutation	• Clinical data (Published, internal) • Tumor data (Published)
MSH2	c.2680dupA (p.M894Nfs*5)	Pathogenic Mutation	• Tumor data (Published, internal)
MSH6	c.4004_4007dupAAGT (p.C1337Sfs*5)	Pathogenic Mutation	• Tumor data (Internal)
PMS2	c.2534delA (p.H845Lfs*6)	Pathogenic Mutation	• Tumor data (internal) • Functional data

RESULTS

- Truncating alterations distal of the NMD boundary were identified in each of the mismatch repair genes: MLH1 (19), MSH2 (16), MSH6 (17) and PMS2 (8) [Fig. 1]
- The most 3' truncating alterations with enough evidence to be classified as pathogenic or likely pathogenic were *MLH1* c.2252_2253delAA (p.K751Sfs*3), *MSH2* c.2680dupA (p.M894Nfs*5), *MSH6* c.4004_4007dupAAGT (p.C1337Sfs*5) and *PMS2* c.2534delA (p.H845Lfs*6) [Fig. 2, Table 1]
- *MSH2* and *MSH6* both have variants of uncertain significance downstream of the mutations reported here, while we have not seen any variants distal of the *MLH1* and *PMS2* mutations [Table 2]
- Although variants have been reported beyond these alterations in available databases, their degree of pathogenicity remains uncertain

Table 2. Truncating Alterations Distal of Known Mutations

Gene	Alteration	Classification	Evidence
MSH2	c.2709delC (p.N903Kfs*4)	VUS	Suspicious clinical history, lacking tumor data, structurally inconclusive
MSH2	c.2785C>T (p.R929*)	VUS	Conflicting tumor data and phenotypes. Co-occurrence with MSH2, MSH6 mutations.
MSH6	c.4016_4017dupCT (p.S1340Lfs*7)	VUS	Limited evidence
MSH6	c.4062_4065dupGACT (p.L1356Dfs*4)	VUS	Published tumor data normal, limited evidence
MSH6	c.4067_4071delTGATT insAATCA (p.L1356*)	VUS	Limited evidence
MSH6	c.4068_4071dupGATT (p.K1358Dfs*2)	Poly	High population frequency

TAKE-HOME POINTS

- Clinical evidence and tumor testing data are essential in determining pathogenicity of truncating alterations in the 3' terminus of genes
- Identifying the most distal known pathogenic mutation would provide evidence to classify other C-terminal truncating alterations
- As evidence used in classifying these alterations may be dependent on unique data from the laboratory, the need for continued data sharing among commercial laboratories is critical

REFERENCES

1. Richards S et al. Genetics in medicine : official journal of the American College of Medical Genetics. 2015;17(5):405-424. doi:10.1038/gim.2015.30.
2. Wang J et al.. EMBO Reports. 2002;3(3):274-279. doi:10.1093/embo-reports/kvf036.