

# Decoding Lynch Syndrome: Resolving difficult to categorize findings in the DNA mismatch repair genes MLH1, PMS2 and MSH2

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## Introduction

Diagnosing Lynch syndrome remains challenging due to limitations in current sequencing methods. In particular, distinguishing pathogenic variants in PMS2 from PMS2CL presents significant diagnostic hurdles; revealing how large deletions affect the expression of MSH2; and determining methylation changes linked to MLH1 expression. This study explores the use of long-read nanopore sequencing to enhance the detection of complex genomic variants.

## Methods

We employed Oxford Nanopore Technologies' PromethION P2 platform with adaptive sampling and the Epi2Me wf-hereditary-cancer pipeline to analyse a comprehensive gene panel (0.9% human genome). This approach enables detection of single nucleotide variants, copy number variation, and epigenetic modifications. Cases included PMS2 mutations, EPCAM deletions and duplications and MLH1 promoter methylation.

## Results

We identified pathogenic PMS2 variants distinct from PMS2CL in 20 patients. resolving inaccessible homologous regions of PMS2CL. Resolution of variants in exon 11, exon 12 and exon 14. Intergenic deletions between EPCAM and the MSH2 promoter were investigated and revealed no change in the methylation profile of MSH2, suggesting that epigenetic control of EPCAM overrides the control of MSH2 expression. Finally, we used Nanopore to interrogate epigenetic change of the promoter region of MLH1 to better define CpG island methylation and its impact on MLH1 expression.

## Conclusion

By addressing the challenges of pseudogenes, repetitive regions, and epigenetic change using long-read sequencing we have shown the diagnostic potential of long read sequencing since it offers a transformative potential in defining difficult to assign mutational events that result in Lynch syndrome. Lynch syndrome is but one example of there long read technology can provide clarity in determining disease risk. Integration of long read sequencing into clinical workflows would reduce diagnostic uncertainty, improve treatment decision-making, and enhance patient outcomes.

Key words: Long-Read Sequencing, Epigenetics, Phasing