PMS2CL-hybrid alleles containing *PMS2* sequence and other *PMS2CL*-derived large rearrangements: The importance of correct interpretation of dosage alteration analysis in *PMS2*

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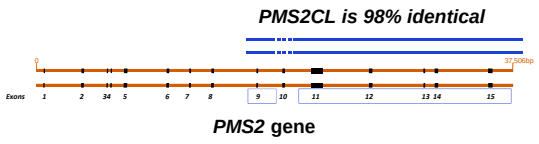
Background

- Approximately 15% of Hereditary Non-Polyposis Colorectal Cancer (HNPCC or Lynch Syndrome) is caused by heterozygous mutations in *PMS2*, with 22% of these mutations categorized as large rearrangements (LRs).
- The interpretation of putative LRs detected in *PMS2* is confounded by frequent and sometimes extensive sequence exchange between *PMS2* and the pseudogene *PMS2CL* within exon 9 and exons 11 through 15.

PMS2 Gene Structure



Adapted from Clendenning et al. *Human Mutation*. 2006;27(5):490-495

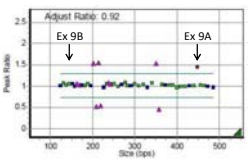
Background

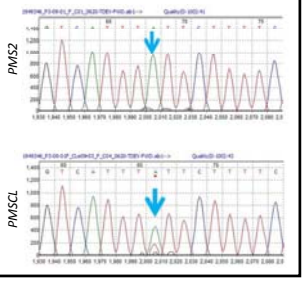
- We have previously observed that 33% of LRs in *PMS2* occur in exons where this gene conversion is common.
- Although multiplex ligation-dependent probe amplification (MLPA) is commonly used for LR analysis of *PMS2*, additional confirmatory analyses are necessary to determine whether LRs are specific to the functional gene.
- Here we demonstrate the necessity of confirmatory analyses to differentiate clinically significant alterations in *PMS2* from benign alterations in *PMS2CL*.

Methods

- Three representative examples of apparent LRs specific to exon 9, exon 11 and exons 13-14 in *PMS2* detected by MLPA or Next Generation Sequencing (NGS) are described.
- These LRs were identified during hereditary cancer testing using a 25-gene panel.
- As part of the panel test, all apparent LRs were further investigated with *PMS2* and *PMS2CL* specific Sanger sequencing and/or long-range PCR analysis.

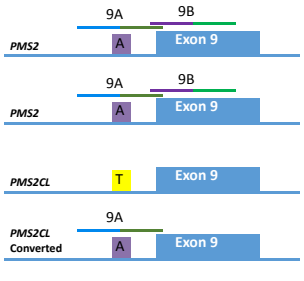
Case 1: Duplication in *PMS2* exon 9?

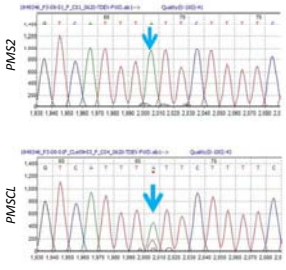




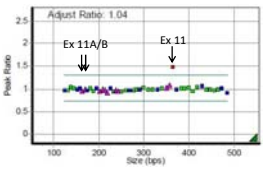
- MLPA shows a duplication in exon 9.
- Confirmatory Sanger sequencing shows that a gene conversion event occurred, which transferred a portion of the sequence from the *PMS2* gene onto *PMS2CL*.

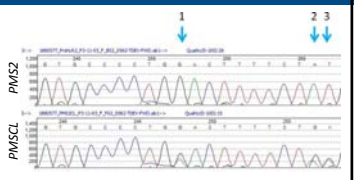
Gene Conversion





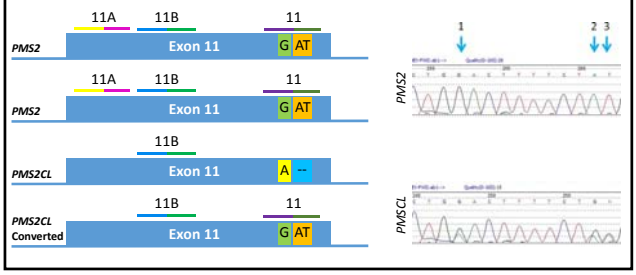
Case 2: Duplication in *PMS2* exon 11?





- MLPA shows a duplication in exon 11.
- Confirmatory Sanger sequencing shows that a gene conversion event occurred, which transferred a portion of the sequence from the *PMS2* gene onto *PMS2CL*.

Gene Conversion



Cases 1 & 2: Interpretation

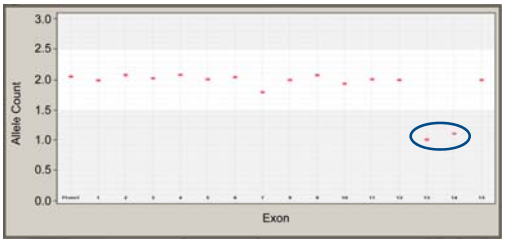
- The gene conversion event occurred, allowing binding of *PMS2*-specific MLPA probes onto the *PMS2CL*-hybrid allele.
- This resulted in the artifactual appearance of a duplication in *PMS2* for both cases.
- Confirmatory Sanger sequencing shows that a gene conversion event occurred, which transferred a portion of the sequence from the *PMS2* gene onto *PMS2CL*.

Cases 1 & 2: Interpretation

Functional/Clinical Interpretation

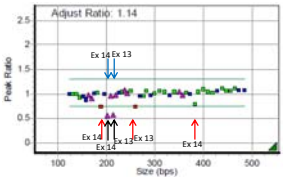
- The *PMS2* gene was not duplicated in these cases/ no clinically actionable large rearrangement variants in *PMS2* exons 9 or 11.

Case 3 NGS : Deletion in *PMS2* exons 13-14?

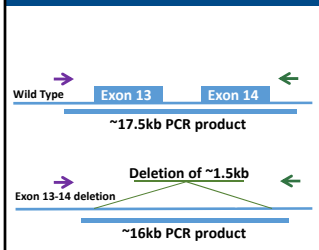


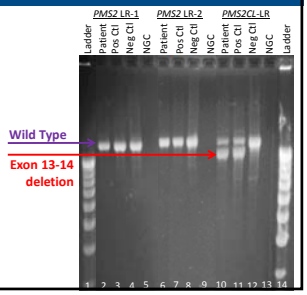
Case 3 MLPA: Deletion in *PMS2* exons 13-14?

- MLPA and NGS show a deletion of exons 13-14.
- Long-range PCR specific to *PMS2CL* and *PMS2* determined that exons 13-14 were only deleted in the pseudogene.



Case 3 Long Range PCR: Deletion in *PMS2CL* exons 13-14





Overall Testing Implications

- The 3 LRs discussed here have been identified in a total of 342 patients, showing the importance of confirmatory analyses for apparent LRs detected in *PMS2*.

Conclusions

- The implementation of a multi-step confirmation strategy allowed for the correct assessment of *PMS2* dosage in the cases described here.
- Accurate interpretation and reporting of *PMS2* is imperative for appropriate medical management and in determining whether testing is recommended for family members.