Clinical Diagnostic *PMS2* Testing in a Single Commercial Laboratory: Challenges in Gross Deletion/Duplication Analysis Due to *PMS2CL* Interference

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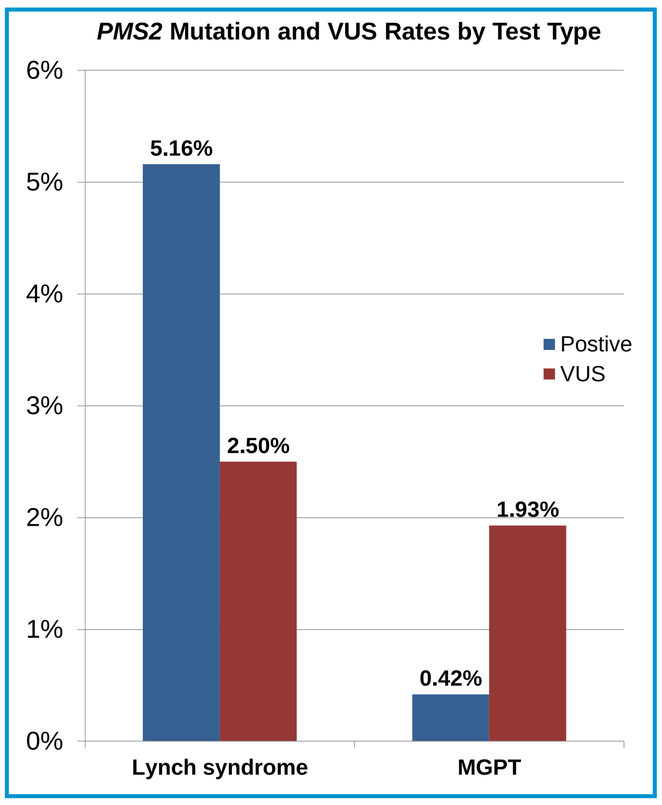
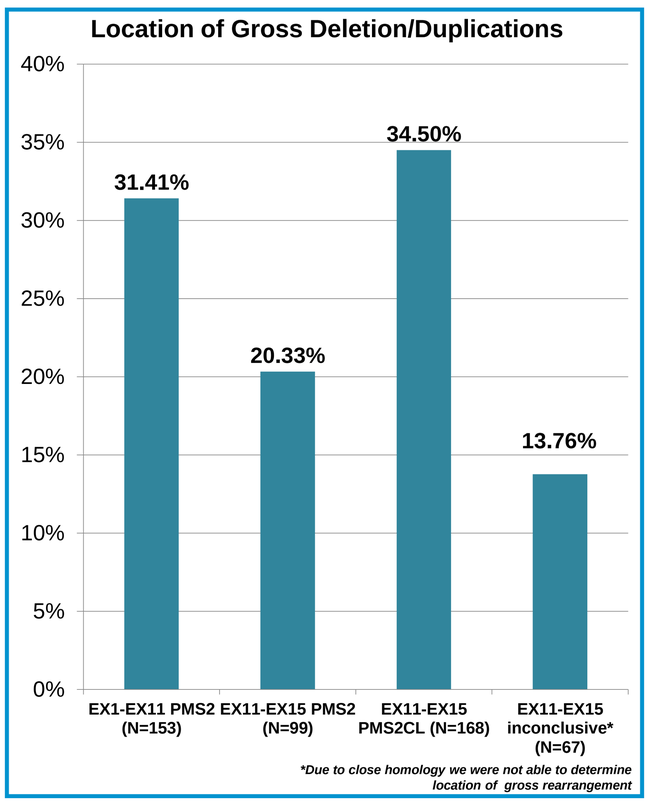
BACKGROUND

- Lynch syndrome is the most common cause of hereditary colorectal cancer and is due to mutations in *MLH1, MSH2/EPCAM*, *MSH6* or *PMS2.*
- Recent data suggests that *PMS2* may account for up to 24% of Lynch syndrome cases1.
- *PMS2* testing is complicated by the presence of pseudogenes. *PMS2CL*, one of the pseudogenes, has close homology to *PMS2* exons 11 to 15 2.
- When alterations are found in this region via NGS and/or MLPA, additional methodologies are necessary to determine if the alteration is in *PMS2* or *PMS2CL*.3
- While these Sanger sequencing protocols can correctly identify the location for single nucleotide variants and small indels, available methodologies are sometimes unable to identify the location of gross deletions/duplications (del/dup) found in exons 11 to 15.
- To better understand the impact of *PMS2CL* interference, we analyzed *PMS2* results in a large cohort of patients undergoing testing for PMS2 either via multi-gene panel testing (MGPT) or Lynch syndrome testing.





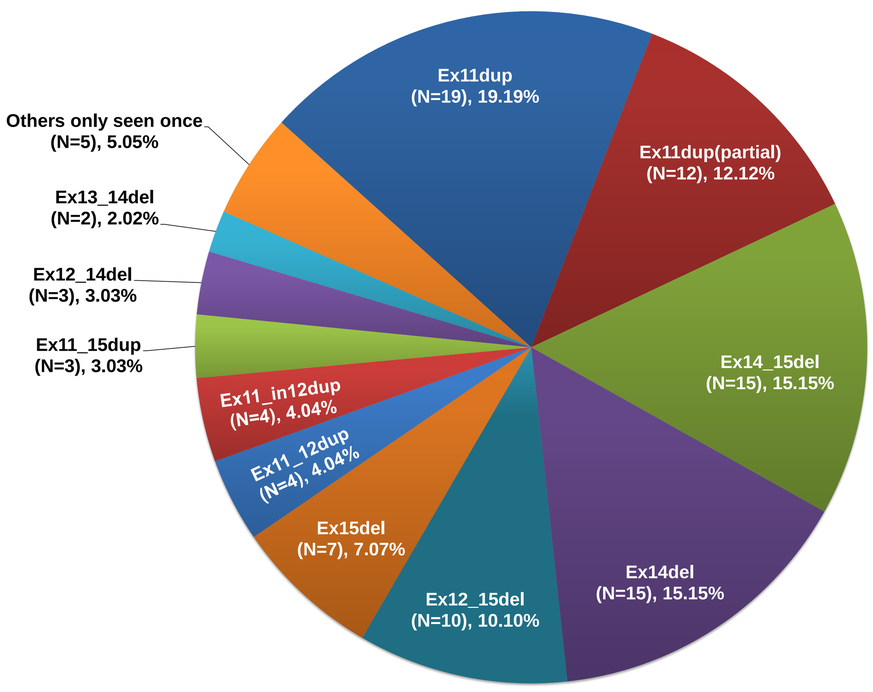
METHODS

- Results of *PMS2* clinical testing via MGPT (N=82,667) or Lynch syndrome testing (N=5,488) from June 2010 through June 2016 at a single laboratory were analyzed.
- Gross del/dup in exons 11 -15 were analyzed in detail to determine the effect of *PMS2CL* interference.

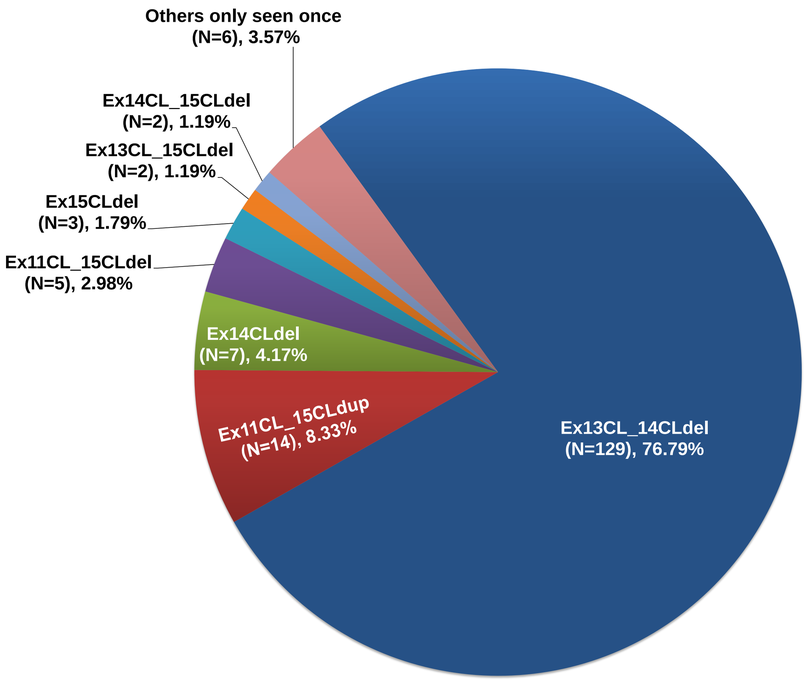
Results of *PMS2* Testing Either via Lynch Syndrome Testing or MGPT

Total # of patients tested via single gene syndrome or MGPT	88,155
Positive	630 (0.07%)
VUS	1664 (1.88%)
Inconclusive	67 (.07%)
Negative	85794 (97.32%)

Distribution of Gross Deletions/Duplications Confirmed to PMS2 Exons 11-15



Distribution of Gross Deletions/Duplications Confirmed to PMS2CL



RESULTS

- Overall positive rate for *PMS2* was 0.07% and VUS rate was 1.88%.
  - Lynch syndrome test positive rate: 5.47%
  - MGPT positive rate: 0.43%
- 153 (0.17%) patients had a del/dup in *PMS2* exons 1-11.
- 334 (0.38%) patients had a del/dup in exons 11-15 requiring follow-up analysis. Using available methodologies, they were assigned as follows:
  - *PMS2* (N= 99, 0.12%)
  - *PMS2CL* (N=168, 0.19%)
  - Inconclusive (N=67, 0.07%): due to close homology.
- The most common del/dups in *PMS2* were Ex11dup (N=19, 19.19%), Ex14_15del (N=15, 15.15%) and Ex14del (N=15, 15.15%), and Ex11dup (partial) (N=12, 12.12%).
- The most common *PMS2CL* del/dup was Ex13CL_Ex14CLdel (N=129, 76.78%).
  - Of these 69.76% were of African/African American descent (90/129).
- 74.62% of the inconclusive results (50/67) were Ex13_14del.

TAKE-HOME POINTS

- The majority (68.58%) of PMS2 gross del/dups occur between exons 11-15 and available methodologies are able to determine the location in a most of these cases: *PMS2* (29.28%) or in *PMS2CL* (50.00%).
- Comprehensive analysis using additional methodologies is necessary when a sequence alteration and/or large del/dup are found between exons 11-15.
- Ex13CL_14CLdel is the most common deletion in *PMS2CL* (76.78%) and may be common in the African population.
- Ex13_14del is also accounts for the majority of difficult to assign cases, due to close homology of *PMS2* and *PMS2CL* and limitations in current methodologies.
- Further studies and development of alternative methodologies are needed to determine the location of a large deletion/duplication located between exons 11-15.

REFERENCES

1. Espenschied, CR, et. al. Multigene Panel Testing Provides a New Perspective on Lynch Syndrome J Clin Oncol 2017 May 17.
2. Nakagawa H, et. al. Mismatch repair gene PMS2: disease-causing germline mutations are frequent in patients whose tumors stain negative for PMS2 protein, but paralogous genes obscure mutation detection and interpretation. Cancer Res 2004 Jul 15;64(14):4721-7.
3. Vaughn CP, et. al. Avoidance of pseudogene interference in the detection of 3' deletions in PMS2. Hum Mutat. 2011 Sep;32(9):1063-71.