PMS2 Pseudogene and Disambiguation: Technically Challenging, Clinically Critical

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Background

Lynch syndrome is characterized by familial predisposition to cancers of the colon, endometrium, ovary, stomach, and urinary tract. Most Lynch syndrome cases are caused by variants in MLH1, MSH2, and MSH6, but 4–11% are caused by variants in PMS2 (with an additional 1% in EPCAM).

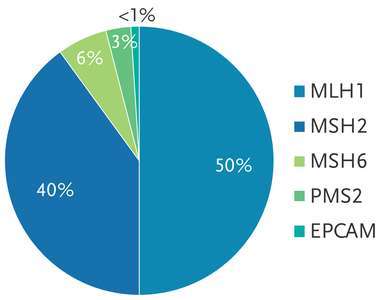


Figure 1: Lynch syndrome gene contribution

PMS2 Cancer Risk

Cancer type	PMS2	General population
Colon cancer	15–20%	5.5%
Endometrial	15%	2.7%
Stomach	6%	<1%
Ovarian	6%	1.6%
Urinary tract	0.4–4%	<1%
Small bowel	0.4–12%	<1%
Pancreas	0.4–4%	1.5%
Brain	1–4%	<1%
Sebaceous neoplasm	Unknown	<1%
Prostate	9–30%	16%
Breast	5–18%	12.4%

Testing for inherited PMS2 variants is hampered by a pseudogene, PMS2CL, which has nearly identical homology to PMS2 in exons 12–15 of the gene. Thus, sequence reads derived from hybridization capture with next-generation sequencing (NGS) cannot be unambiguously aligned to PMS2 or PMS2CL. Gene conversion within PMS2 exons 12–15 and PMS2CL exons 3–6 further complicates this issue.

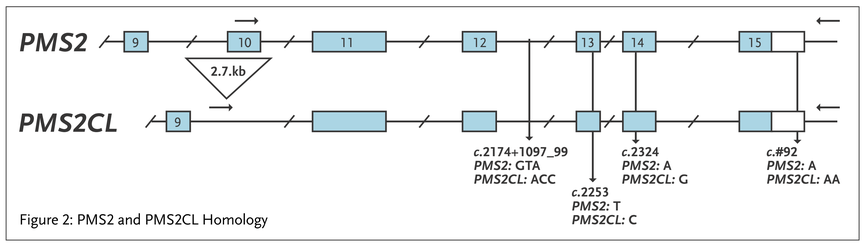


Figure 2: PMS2 and PMS2CL Homology

Methods:

Internal data from samples tested for PMS2 were collected from August 2015 until June 2016 for full analysis of PMS2 exons 12–15. Analysis of this region includes the "gold standard method" described in Vaughn CP, et al. 2011as well as a novel, validated approach to disambiguating variants in this region.

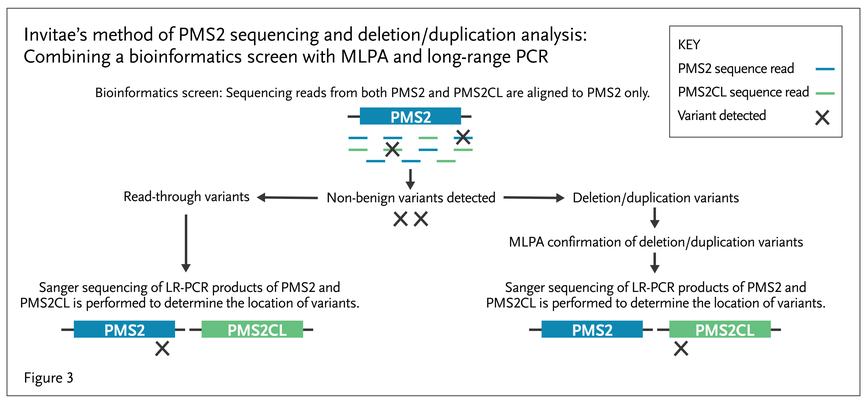


Figure 3

Results from 250 patients with variants in PMS2 exons 12–15 or PMS2CL exons 3–6 were analyzed. A subset analysis of the clinical attributes of 44 patients with likely pathogenic or pathogenic PMS2 (exons 12–15) results was performed with data from ordering clinicians.

Results

Of 265 total variants in PMS2 exons 12-15, 44 are in PMS2 and 220 are in PMS2CL, and 1 duplication could not be disambiguated.

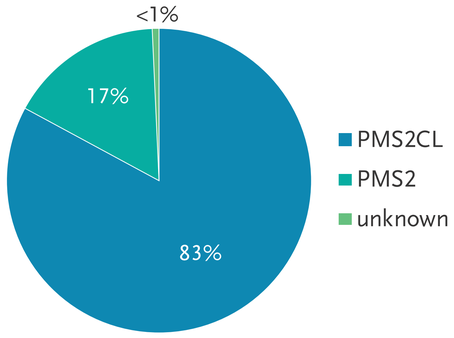


Figure 4: Distribution of PMS2 and PMS2CL variants post disambiguation

Summary of Variants in PMS2 exons 12-15

PMS2CL	PMS2	Variant
108	1	c.2186_2187delTC
105	1	c.2243_2246delAGAA
2	8	Deletion (Exon 14)
0	7	c.2117delA
0	6	Duplication (Exon 11–12)
2	3	c.2192_2196delTAACT
0	1	c.2445+1G>T
0	4	Deletion (Exons 14–15)
0	3	Deletion (Exons 3–15)
0	5	Deletion (Exon 12)
1	3	Deletion (Exon 11–15)
0	1	Whole Gene Deletion
1	0	Deletion(Exons 12–15)
0	1	Deletion (Exons 9–15)

A particular variant cannot be used solely to distinguish PMS2 from PMS2CL. Example: deletion of exon 14 was observed multiple times in the pseudogene and PMS2.

Adding NGS technology increases disambiguation yield compared to "gold standard method" alone in deletion/duplication calls

49 del/dup calls in exons 13-14

MLPA and LR-PCR/Sanger = 36/49 = 73.47%

NGS, MLPA, and LR-PCR/Sanger = 48/49 = 97.96%

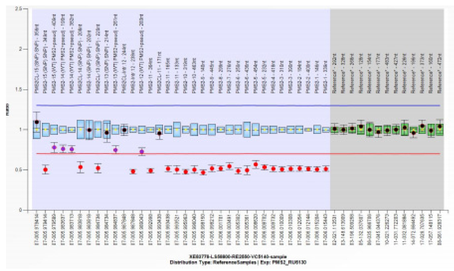


Figure 5a: MLPA data- whole gene deletion

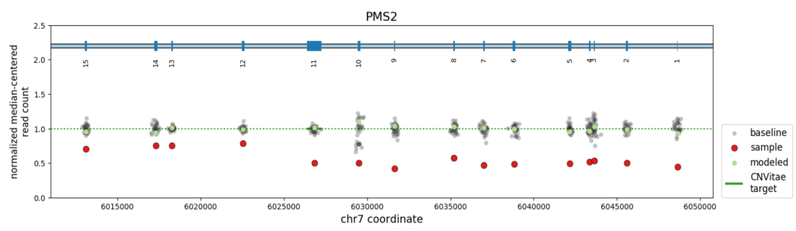


Figure 5b: NGS- whole gene deletion

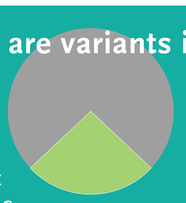
Results-Of Clinical Interest

26/44 PMS2 Pathogenic or Likely Pathogenic met professional testing guidelines

- Several with no personal or family history of LS cancers
- Only 1 met Amsterdam criteria

How common are variants in this region?

26% of Invitae's pathogenic and likely pathogenic PMS2 variants were in exons 12–15



PMS2 is low penetrance and therefore not all cases have a striking family history. For patients outside criteria, most PMS2 variants were detected through **larger cancer panels**.

40% of these patients did not meet the criteria for Lynch syndrome testing

Discrepant Family Variant Testing

Case #1

- Relative was found to have a deletion in exons 1-10 at another lab.
- Testing our patient found deletion of entire PMS2 gene by MLPA, RT-PCR and CNV
- Methods at original lab were aCGH
  - "large deletions or duplications in PMS2 exons 2-5, 9, and 11-15 will not be detectable due to the presence of pseudogenes"

Case #2

- Relative was found to have PMS2 pathogenic variant at outside lab
- Testing our patient- Familial PMS2 variant was mapped to the pseudogene (PMS2CL)

Conclusions

Our methods disambiguated more than 99% of pathogenic or likely pathogenic variants detected in PMS2 exons 12–15. Germline genetic results are key factors in diagnosing Lynch syndrome. Only 40% of patients had clinical features consistent with the syndrome, whereas others were diagnosed mainly due to ordering large panels in non-Lynch cancer families. Of all the total PMS2 pathogenic and likely pathogenic variants during this time frame, 26% were in exons 12-15 suggesting that variants in this region are not uncommon. Variant interpretation in this region of PMS2 is highly dependent on laboratory methods. Therefore, a laboratory's technology and methods are crucial in avoiding misdiagnoses of Lynch syndrome and inappropriate management.

References

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