




BRIEF REPORT

PMS2CL interference leading to erroneous identification of a pathogenic *PMS2* variant in Black patients



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ABSTRACT

This study investigates the frequency of a clinically reported variant in *PMS2*, NM_000535.7:c.2523G>A p.(W841*), from next-generation sequencing studies in 2 racially diverse cohorts. We identified clinical reports of the *PMS2* c.2523G>A p.(W841*) variant in the National Precision Oncology Program's somatic testing database ($n = 25,168$). We determined frequency of the variant in germline exome sequencing from the Penn Medicine BioBank ($n = 44,256$) and in gnomAD. The *PMS2* c.2523G>A p.(W841*) was identified as a homozygous variant on tumor testing in an adult patient of self-identified Black race/ethnicity with no evidence of constitutional mismatch repair deficiency. The variant was clinically reported on 35 total tumor and liquid biopsy tests (0.1%), and all individuals with the variant were of self-identified Black race/ethnicity (0.6% of $n = 5787$). In individuals of African genetic ancestry (AFR), the variant's germline frequency was reported to be 0.2% and 1.3% in the Penn Medicine BioBank (PMBB) and gnomAD, respectively. The variant cannot be found in any individuals of European genetic ancestry (EUR) from either of the databases. The variant is found in a region of *PMS2* with 100% homology to the *PMS2CL* pseudogene. *PMS2* c.2523G>A p.(W841*), when identified, is typically an African-ancestry-specific *PMS2CL* pseudogene variant, which should be recognized to prevent misdiagnosis of Lynch syndrome in Blacks.

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Introduction

Lynch syndrome (LS, OMIM 614337) is a common hereditary cancer syndrome associated primarily with increased risks for colon and endometrial cancer. Germline variants in mismatch repair genes *MLH1*, *MSH2*, *MSH6*, *PMS2*, and *EPCAM* cause LS.¹ Diagnosis of LS occurs through germline genetic testing, although the analysis of tumor tissue through mismatch repair immunohistochemistry can aid in identifying suspected LS in cancer patients.¹ Similarly, somatic genetic testing can also play an important role in detecting hereditary cancer predisposition syndromes by revealing germline variants, in addition to acquired somatic variants.² Variant allele frequency (VAF) can be a telling indicator of germline variants identified in the somatic setting.³ When using short-read methods for germline or somatic testing, it is possible that pseudogenes can interfere with sequencing analysis, specifically read mapping; this is especially difficult for genes such as *PMS2* (HGNC:9122) that have several known pseudogenes such as *PMS2CL* (HGNC:30061).^{4,5} Herein, we describe a *PMS2* variant that was reported as pathogenic on somatic tumor testing only in individuals of self-reported Black race/ethnicity (Black). This variant was determined to be a *PMS2CL* pseudogene variant and confirmed to be carried at a higher frequency by individuals of self-reported Black race/ethnicity or genetically confirmed African ancestry. Recognition of this pseudogene interference is important to prevent the erroneous diagnosis of LS.

Materials and Methods

Durham VA Healthcare System Institutional Review Board approved the study. Informed consent was obtained for all patient volunteers who donated blood samples to the Penn Medicine BioBank (PMBB) for exome sequencing (ES) at median 65× coverage.^{6,7} National Precision Oncology Programs (NPOP) somatic testing database is an operational database containing identified information from patients tested through NPOP.^{8,9} We identified clinical reports of the NM_000535.7:c.2523G>A p.(W841*) variant in *PMS2* in the NPOP somatic testing database ($n = 25,168$). We determined frequency of the variant in germline ES from the PMBB ($n = 44,256$) and compared this with the reported population frequency in gnomAD (v2.1.1).¹⁰

Results

In an effort to identify patients with putative *PMS2*-related LS by tumor-only testing, the NPOP database ($n = 25,156$ patients) was queried and 156 reports with likely pathogenic/pathogenic (LP/P) variants in *PMS2* were identified. Eighty-six different *PMS2* LP/P variants were identified. Five variants were seen in 5 or less tumors, but 1 variant, a

PMS2 variant denoted as “p.(W841*),” was seen in 35 cases (*PMS2* c.2523G>A). Among these tumors, a breast tumor with microsatellite stable (MSS) status and a tumor mutational burden of 0 mutations/megabase was identified where the variant was reported at an allele fraction of 100%. Given the peculiarity of the frequency of this variant and the absence of an expected MS instability signature in a tumor with VAF 100%, we investigated the variant in more detail.

Upon query to the tumor testing company, the Human Genome Variant Society nomenclature of the identified variant was c.2523G>A p.(W841*). Query of ClinVar revealed 2 separate cDNA changes, *PMS2* c.2522G>A (ClinVar ID: 234759) and *PMS2* c.2523G>A (ClinVar ID: 127786), which correspond to the same predicted protein truncation reported on the somatic testing, *PMS2* p.(W841*). ClinVar had several submissions by commercial germline genetic laboratories under *PMS2* c.2522G>A that were classified as pathogenic. For *PMS2* c.2523G>A, ClinVar had 1 entry from a commercial germline genetic testing labs that deemed this variant pathogenic; whereas, several smaller labs labeled this a variant of uncertain significance. Germline genetic testing was carried out on blood lymphocytes from the patient with the tumor variant at VAF 100% using a large commercial laboratory. Despite the high VAF, the patient was not identified to carry any LP/P variant in *PMS2* in the germline. The laboratory was contacted, and a data review was requested. Upon review, the commercial laboratory confirmed that germline *PMS2* c.2523G>A p.(W841*) was not present in *PMS2*, but a variant was confirmed at the homologous position of the *PMS2CL* pseudogene by long-range polymerase chain reaction (PCR). The *PMS2CL* variant was homozygous. The lab confirmed that the analysis was not confounded by allelic dropout nor coamplification.

In the NPOP database, the *PMS2* c.2523G>A variant was reported in 22 patients out of 19,196 (0.11%) solid tumor samples that were successfully sequenced and reported via Foundation One CDx (Table 1). All 22 patients were of Black self-identified race/ethnicity. Microsatellite status was available for 21 of the 22 patients, with 19 of the 21 (90%) having MSS tumors, and 2 of the 21 (10%) were equivocal. Comparatively, 94 other tumors with truncating variants in *PMS2* (Supplemental Table 1) and MS status available were analyzed, and 34 of the 94 (36%) were MS unstable (MSI-High) (Supplemental Figure 1A). Reviewing liquid biopsy reports, the c.2523G>A variant was reported in 2 patients out of 2990 (0.07%) liquid biopsy samples that were successfully sequenced and reported via Foundation One Liquid CDx (Table 1). Both patients who self-identified as Black and MS status were unavailable. Finally, the *PMS2* c.2523G>A variant was reported in 11 patients out of 2982 (0.37%) solid tumor samples sequenced via the Personal Genome Diagnostics CP6 tumor-only test, with all 11 patients having self-identified Black race/ethnicity (Table 1). The *PMS2* c.2523G>A variant was not identified in the 1361 NPOP patients who had tumor sequencing through 1396 Personalis tests (data not shown). The variant call file data were reviewed, and the reported *PMS2* p.(W841*)

Table 1 Frequency of *PMS2* c.2523G>A; p.W841X in a tumor testing cohort, germline exome sequencing study, and population databases

Cohort	All Patients			SIRE-Black/AFR ^a			SIRE-White/EUR ^a			Other ^a			# of Homo-zygotes
	<i>n</i> POS	<i>n</i> Total	%	<i>n</i> POS	<i>n</i> Total	%	<i>n</i> POS	<i>n</i> Total	%	<i>n</i> POS	<i>n</i> Total	%	
NPOP FoundationOne-CDx	22	19196	0.11%	22	4170	0.53%	0	13642	-	0	1384	-	1 (SIRE-Black)
NPOP FoundationOne-LiquidCDx	2	2990	0.07%	2	886	0.23%	0	1920	-	0	184	-	None
NPOP PGDX CP6	11	2982	0.37%	11	731	1.50%	0	2141	-	0	110	-	None
<i>NPOP Database (total)</i>	<i>35</i>	<i>25168</i>	<i>0.14%</i>	<i>35</i>	<i>5787</i>	<i>0.60%</i>	<i>0</i>	<i>17703</i>	-		<i>1678</i>		
Penn Medicine BioBank	28	44256	0.06%	25	10815	0.23%	0	29239	-	3	4112	0.07%	None
GnomAD	97	96159	0.10%	91	7106	1.28%	0	41415	-	5	47998	0.01%	3 (AFR)

POS, number of cases with an identified *PMS2* p.W841X variant; *SIRE*, self-identified race/ethnicity.

^aPatients were categorized by self-identified race/ethnicity (*SIRE*) as SIRE-Black or SIRE-White or “Other” using the Veterans’ Affairs electronic health record for NPOP data. In the Penn Medicine BioBank and gnomAD, samples were categorized as “AFR,” “EUR,” or “Other” by genetic informed ancestry analysis.

variants were the c.2523G>A allele in all cases. The *PMS2* c.2522G>A variant was not identified in any of the NPOP patients. Overall, the *PMS2* c.2523G>A p.(W841*) was reported in 35 (0.60%) of 5787 Black individuals and none of 17,703 White individuals in the NPOP database (Table 1). Of the 35 tumor types with *PMS2* p.(W841*) reported, 28% were LS-related cancers; although this was not much lower than the rate of LS-related cancers in other truncating variants in *PMS2* (36% of 121 cases) (Supplemental Figure 1B).

We next investigated research ES data from 44,256 patient samples in the PMBB. ES was performed at median 65× coverage. The *PMS2* c.2523G>A p.(W841*) variant was identified overall in 28 individuals (0.06%) (Table 1). Of the 28 individuals, 25 were of African genetic-inferred ancestry (89.3%), and 3 were of other non-White genetic ancestral background (10.7%). The frequency of the *PMS2* c.2523G>A in the African genetic ancestry population in PMBB was 0.23%. This is lower than the reported variant frequency in gnomAD, which reports the overall population frequency at 0.10% and the African genetic ancestry population frequency at 1.3% (Table 1).¹⁰

Finally, 3 commercial laboratories were contacted to review the evidence for their classifications. The 2 commercial labs that had submitted P calls under *PMS2* c.2522G>A p.(W841*) noted that *PMS2* c.2522G>A p.(W841*) had been identified by next-generation sequencing (NGS) in only a few patients, and long-range PCR confirmed that it was located in the *PMS2* gene. Conversely, *PMS2* c.2523G>A p.(W841*), as was observed in this study, had been identified by NGS in over 200 patients at each lab and had never been confirmed in *PMS2* by long-range PCR, with each lab concluding that *PMS2* c.2523G>A p.(W841*) was instead located in the pseudogene *PMS2CL*. The third commercial laboratory who had submitted a pathogenic call under *PMS2* c.2523G>A p.(W841*) on ClinVar noted that this variant had never been confirmed in *PMS2* by long-range PCR.

This laboratory marked *PMS2* c.2523G>A p.(W841*) as an artifact in their pipeline, with identification of this variant by NGS always being followed up with long-range PCR. The laboratory’s justification for their pathogenic ClinVar submission was that a truncating variant in that position would be pathogenic, if confirmed to be in *PMS2* by long-range PCR. Review of DNA alignment of *PMS2* exon 15 and *PMS2CL* exon 6 showed 100% homology, with a substitution of G>A in codon 841 of *PMS2* creating a stop codon (nucleotides TAG) in both the pseudogene and functional *PMS2* (Figure 1), explaining the pseudogene interference at this position.

Discussion

Pseudogenes are copies of functional genes that exist within the genome that do not serve as DNA templates for functional protein products. These pseudogenes can be highly similar to the functional gene across large stretches of base pairs.⁴ Although NGS has provided a high-throughput, efficient, and cost effective way of performing DNA sequencing, this method depends on short reads, which can make hybrid capture, read mapping, and subsequent variant calling susceptible to pseudogene interference.⁴ Short reads may not include areas of the gene that are distinct from the pseudogene and therefore could inadvertently capture the pseudogene sequence.⁴

PMS2 and the *PMS2CL* pseudogene contain highly similar sequences in exons 9 and 11 to 15.^{4,5} If using short-read sequencing, the high degree of similarity between *PMS2* and *PMS2CL* in several adjacent exons makes this region highly susceptible to pseudogene interference. Long-read methods such as long-range PCR should be used to capture outside of the homologous regions to distinguish variants identified in *PMS2CL* from variants in *PMS2*.⁴

The *PMS2* c.2522G>A p.(W841*) variant is located in exon 15, the last exon of the *PMS2* gene. Exon 15 is associated with the nuclease domain, and deletions in exon 15 damage this

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|PMS2 exon 15 TGATGATTGGGACTGCTCTTAACACAAGCGAGATGAAGAAACTGATCACCCACATGGGGG 60
  |||
PMS2CL exon 6 TGATGATTGGGACTGCTCTTAACACAAGCGAGATGAAGAAACTGATCACCCACATGGGGG 2201

|PMS2 exon 15 AGATGGACCACCCCTGGAACTGTCCCCATGGAAGGCCAACCATGAGACACATCGCCAACC 120
  |||
|PMS2CL exon 6 AGATGGACCACCCCTGGAACTGTCCCCATGGAAGGCCAACCATGAGACACATCGCCAACC 2261

|PMS2 exon 15 TGGGTGTCATTTCTCAGAACTGA 143
  |||
PMS2CL exon 6 TGGGTGTCATTTCTCAGAACTGA 2284

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Figure 1 *PMS2* exon 15 aligned with *PMS2CL* exon 6. Illustrates the similarity in *PMS2* exon 15 and *PMS2CL* exon 6 sequences. Highlighted in red is the codon corresponding to the variants of interest *PMS2* c.2523G>A p.(W841*).

nuclease domain that is critical for *PMS2* function.¹¹ Other downstream variants are P and LP (ClinVar). Variants in exon 15 of *PMS2* may be difficult to distinguish from the pseudogene because exon 6 of *PMS2CL* is identical.¹² The pseudogene variant c.2523G>A p.(W841*) has been reported in several articles that all fail to differentiate this variant in *PMS2CL* versus *PMS2*.^{13–17} Because exon 6 of *PMS2CL* is identical to exon 15 of *PMS2*, a substitution of G>A in codon 841 creates a TAG stop codon in both the pseudogene and functional *PMS2*. Short-read sequencing could inadvertently capture the truncating *PMS2CL* variant while attempting to sequence *PMS2*.

The *PMS2* variant p.(W841*) must be distinguished by cDNA change. *PMS2* c.2522G>A is a pathogenic truncating variant truly located in the *PMS2* gene that has been seen infrequently by commercial laboratories and is consistent with LS. On the other hand, *PMS2* c.2523G>A is a single-nucleotide variant (SNV) in the pseudogene *PMS2CL* commonly found in the African American population. It is important for clinicians, germline and somatic genetic testing laboratories, and bioinformaticians to recognize *PMS2* c.2523G>A as a pseudogene SNV to prevent misdiagnosis of LS in Black patients carrying the SNV. This pseudogene variant has been confused for a pathogenic *PMS2* variant in the literature and is also present in population databases such as gnomAD as a true *PMS2* variant.¹⁰ Of note, cBioportal and AACR GENIE have not included the pseudogene variant—possibly because their bioinformatics team excluded it.^{18,19} This pseudogene variant c.2523G>A should be regarded as an artifact in prospective cases and should be excluded from population databases and germline and somatic genetic testing reports to limit confusion with the pathogenic *PMS2* variant c.2522G>A.

Data Availability

The data are available by contacting the corresponding author. The data are available to qualified investigators with access to VA's VINCI computing environment and an approved IRB.

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Ethics Declaration

Durham VA Healthcare System Institutional Review Board approved the study. Obtained informed consent for all patient volunteers who donate biological samples to the Penn Medicine BioBank.^{13,14} <https://pmbb.med.upenn.edu>. National Precision Oncology Programs somatic testing database is an operational database containing identified information from patients tested through NPOP.^{15,16}

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Conflict of Interest

The authors declare no conflicts of interest.

Additional Information

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