Over-Representation of *PMS2CL* Pseudogene Interference in *PMS2* Testing in a Non-European Cohort

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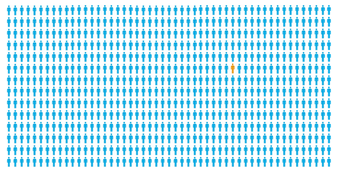
Background

Lynch syndrome, associated with pathogenic variants in *MLH1, MSH2/EPCAM, MSH6, PMS2,* or *EPCAM*, is the most common cause of hereditary colorectal cancer, additionally predisposing to uterine, ovarian, prostate, and other cancers.

Approximately 1 in 714 individuals in the general population have a ***PMS2*** pathogenic variant.[1]



Homology between *PMS2* exons 11-15 and its processed pseudogene *PMS2CL* presents challenges for genetic testing laboratories utilizing next-generation sequencing. Misclassifications have occurred, including in non-European cohorts:

Insertion/deletion (indel) c.2182_2184delACTinsG, common in individuals of African ancestry in the general population, was resolved in some patients to be in *PMS2CL*.[2]

Same *PMS2CL* indel (left) was identified in a Brazilian population, having been misclassified as pathogenic variants in *PMS2*.[3]

Single nucleotide variant (SNV) c.2523G>A (p.W841*), reported as pathogenic on tumor testing, was determined to be in *PMS2CL* in individuals of African ancestry.[4]

Existing methodologies (e.g., long-range PCR [LR-PCR] and Sanger sequencing) can clarify location of SNVs or indels if utilized.

Gross deletions/duplications (del/dups) are not always reliably resolved.

In one study examining impact of *PMS2CL* interference in *PMS2* analysis, the most common *PMS2CL* del/dup (Ex13_Ex14del) appeared to be over-represented in individuals of African descent.[5]

This preliminary study investigated cases of del/dups requiring follow-up testing to resolve to *PMS2* or *PMS2CL* at one commercial laboratory, aiming to determine if there is disproportionate ambiguity by ancestry.

Methods

Retrospective review of cases including *PMS2* testing:

Performed between 06/2023-11/2024 at one commercial genetic testing laboratory

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Potential del/dup within *PMS2* exons 11-15 warranting follow-up testing to clarify location in *PMS2* or *PMS2CL* [MLPA (multiplex ligation-dependent probe amplification), +/- LR-PCR with sequencing and/or gel electrophoresis]

Reported ancestry categorized as:

"European"
- "White"
- "Ashkenazi Jewish"

Excluded
- "Unknown"
- "Other"
- "Mixed Ethnicity"

"Non-European":
- "African American/Black"
- "Alaskan Native"
- "Asian"
- "Hispanic"
- "Middle Eastern"
- "Native American"
- "Pacific Islander"

Statistical analyses were performed via chi-square test.

Strategies for *PMS2* analysis at other germline genetic testing laboratories were ascertained via discussion with laboratory representatives as well as review of publicly available resources.

National Society of Genetic Counselors

Results

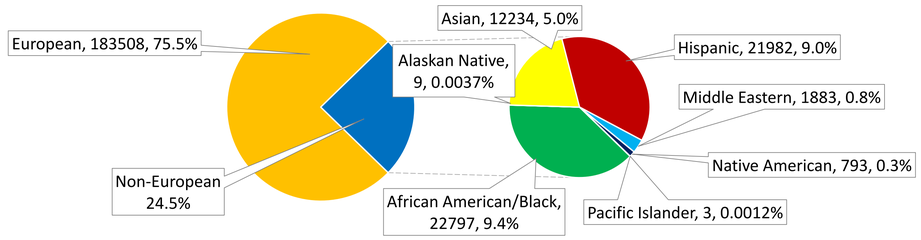

Figure 1: Reported ancestry from all cases which included *PMS2* testing.

Among 243,209 cases, 24.5% (n=59,701) of individuals were of non-European ancestry, most commonly African American/Black (n=22,797; 9.4%), Hispanic (n=21,982; 9.0%), or Asian (n=12,234; 5.0%).

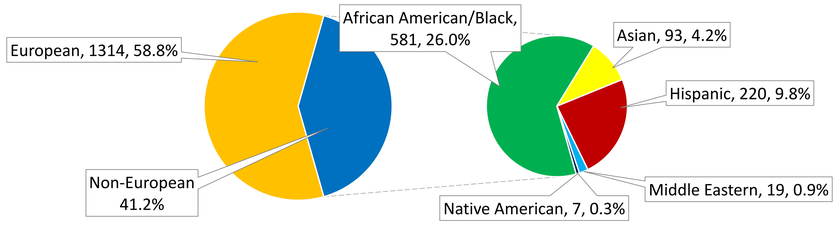

Figure 2: Ancestry from cases requiring follow-up testing to resolve del/dup in *PMS2* or *PMS2CL*

Among cases of del/dups requiring follow-up (n=2,234), non-European cases were overrepresented (see analyses below): specifically, one quarter (n=581, 26.0%) were in the African American/Black group, or 63.2% of these non-European cases.

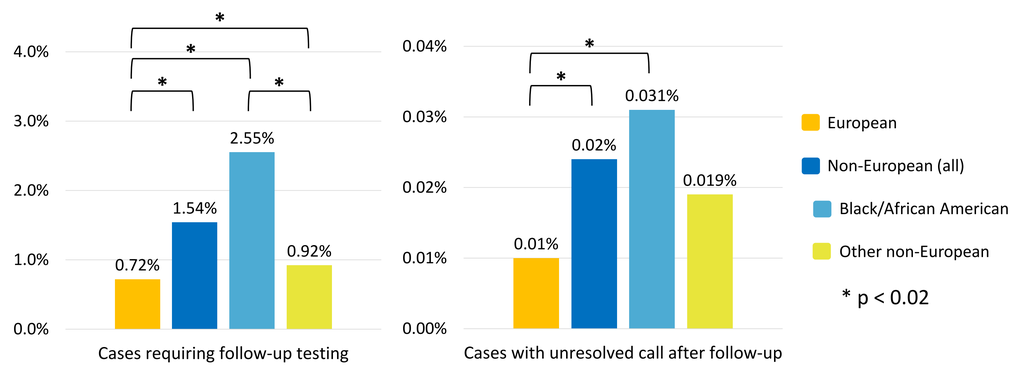

Figure 3: Ancestry differences in outcomes of follow-up to resolve del/dups within *PMS2* exons 11-15 or *PMS2CL*

Among all *PMS2* testing, non-European cases were twice as likely than European cases to have a call requiring follow-up or to ultimately have a result with an unresolved *PMS2* call. This difference was greater for those with African American/Black ancestry. Most cases requiring follow-up testing were resolved, with no significant difference in rate of resolution by ancestry (97.9-98.8%).

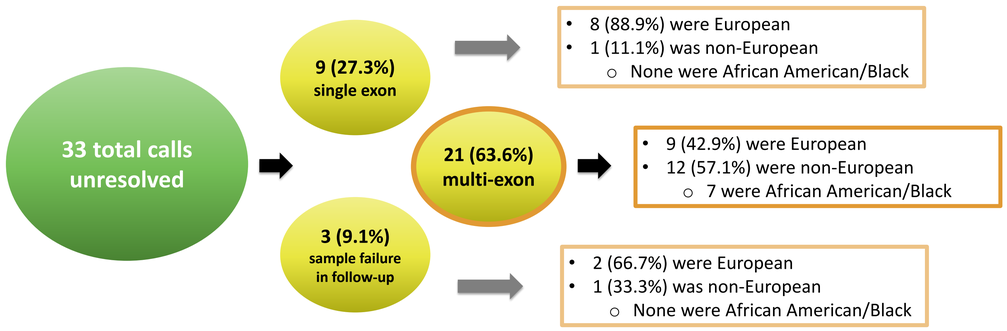

Figure 4: Types of variants remaining unresolved after follow-up testing by ancestry

Nearly half (8/18, 44.4%) of germline genetic testing laboratories contacted via email provided information about available techniques for *PMS2* analysis.

Available techniques for *PMS2* follow-up vary, including long-range PCR +/- MLPA, long-read sequencing, and/or laboratory-specific data analysis protocols.

Where information was ascertained, specifications such as factors informing initiation of follow-up analysis or figures on technique efficacy were not consistently available.

Discussion

- Technical limitations of next-generation sequencing of *PMS2,* given homology with pseudogene *PMS2CL*, have the potential to contribute to disparities related to ancestral background.
- Non-European cases, especially those with African American/Black ancestry, were overrepresented among cases with a del/dup that either required follow-up testing to resolve variant location or ultimately had a result with an unresolved call after follow-up.
- All (7/7) unresolved calls within the African American/Black group were multi-exon. Some or all could represent the common *PMS2CL* del/dup (Ex13_Ex14del), though unable to be confirmed.
- Existing methodologies for *PMS2* follow-up analysis used by this laboratory appear to sufficiently differentiate most del/dups within this region, regardless of reported ancestry.
- Information about protocols at germline genetic testing laboratories in general, or rate of variant call resolution, was not reliably available.
- Future directions include partnerships with additional germline genetic testing laboratories to evaluate for evidence of potential disparities and encourage uptake of methodologies which improve health equity.

References

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