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

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Introduction: Lynch syndrome (associated with pathogenic variants in *MLH1*, *MSH2*, *MSH6*, *PMS2*, or *EPCAM*) is the most common cause of hereditary colorectal cancer. Approximately 1 in 714 individuals in the population have a *PMS2* pathogenic variant. Homology between *PMS2* exons 11-15 and its processed pseudogene *PMS2CL* presents challenges for genetic testing laboratories. Misclassifications have occurred, with common African *PMS2CL* single nucleotide variants and indels erroneously assigned to *PMS2* and classified as pathogenic. While existing methodologies (e.g., long-range PCR [LR-PCR] and Sanger sequencing) can resolve the location of these variants, gross deletions/duplications (del/dups) are not always reliably resolved. *PMS2CL* Ex13CL_14CLdel is a commonly identified variant over-represented in individuals of African descent. It is unclear whether additional differences by ancestry exist in prevalence or rate of resolving these variants. This preliminary study aimed to investigate cases of del/dups requiring follow-up testing to resolve location in *PMS2* or *PMS2CL*, with focus on reported ancestry, to determine if there is disproportionate ambiguity in non-European cohorts.

Methods: Retrospective review was performed for cases including *PMS2* testing between 06/2023-11/2024 at one commercial genetic testing laboratory, with focus on those where a potential del/dup within exons 11-15 warranted follow-up testing to clarify location as *PMS2* or *PMS2CL*. Follow-up involved MLPA, +/- LR-PCR followed by sequencing and/or gel electrophoresis. Reported ancestry was categorized as “European” (“White” or “Ashkenazi Jewish”) or “non- European” (“African American/Black,” “Alaskan Native,” “Asian,” “Hispanic,” “Middle Eastern,” “Native American,” or “Pacific Islander”). Those categorized as “Unknown,” “Other,” or “Mixed Ethnicity” were excluded from analysis. Statistical analyses were performed via chi-square test.

Results: Among 243,209 cases including *PMS2* testing, 24.5% (n=59,701) individuals were of non-European ancestry, most commonly African American/Black (9.4%), Hispanic (9.0%), or Asian (5.0%). In total, 0.92% (n=2,234/243,209) of cases were identified to have a potential del/dup within *PMS2* exons 11-15 or its homologous region in *PMS2CL* requiring follow-up; those in the non-European group were twice as likely than those in the European

group to have a *PMS2* call requiring follow-up [non-European, 1.54% (n=920/59,701); European, 0.72% (n=1,314/183,508)], a statistically significant difference in prevalence by ancestry, χ^2 (1, N = 243,209) = 336.85, p = 3.10×10^{-75}. More than half, 63.2% (n=581/920), of individuals in the non-European group with a *PMS2* call requiring follow-up were Black/African American. Among cases with follow-up testing, 1.52% (n=14/920) in the non-European group remained unresolved compared to 1.45% (n=19/1,314) in the European group; there was no statistically significant difference in rate of resolution by ancestry, χ^2 (1, N = 2,234) = 0.021, p = 0.88.

However, among all individuals who underwent *PMS2* testing, those in the non-European group were twice as likely than those in the European group to have an unresolved *PMS2* call [non-European, 0.024% (n=14/59,701); European, 0.010% (n=19/183,508)], a statistically significant difference, χ^2 (1, N = 243,209) = 5.69, p = 0.017.

Conclusion: This study highlights how technical limitations of next-generation sequencing, potentially relevant across genetic testing laboratories, can contribute to disparities related to ancestral background. Gross del/dups within exons 11-15 of *PMS2* or its homologous region in *PMS2CL* that required follow-up, or which remained unresolved after follow-up, were over-represented in the non-European group. More than half of non-European calls were in Black/African American individuals, possibly due to the common *PMS2CL* Ex13CL_Ex14CLdel. Existing methodologies for follow-up analysis used by this laboratory appear to sufficiently differentiate the majority of del/dups within this region, regardless of reported ancestry. However, it is not known whether these approaches are consistently utilized across different genetic testing laboratories. Uptake of reliable methodologies can inform both clinical validity and utility of genetic testing in order to increase health equity.