Application of RNA Sequencing Evidence Improves Equity in Variant Interpretation

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Background: Disparities in genetic test results for hereditary cancer predisposition are well known, whereby a higher rate of variants of uncertain significance (VUS) and a lower rate of pathogenic variants (PV) are identified in racial and ethnic minorities compared to non-Hispanic white (NHW) individuals. Lack of representation of non-white and Hispanic individuals in testing cohorts, published literature and population databases contributes to this disparity, as it limits the availability of evidence used towards variant classification (i.e., case-control data, proband counting co-segregation, population frequency). However, functional data generated by clinical tests that include RNA sequencing (paired DNA/RNA genetic testing) create the potential for an additional line of evidence for all individuals tested. In this study, we compared the impact RNA evidence had on test results across a variety of racial and ethnic groups. Based on the known gaps in evidence for racial and ethnic minorities, we hypothesized that the additional line of evidence generated by RNA sequencing would preferentially improve variant classification in non-whites compared to non-Hispanic whites.

Methods: Demographics and test results for individuals who underwent paired DNA and RNA genetic testing for hereditary cancer predisposition from March 2019 through April 2020 were retrospectively reviewed. We evaluated variant classification, VUS rate, and PV rate with and without RNA evidence amongst individuals who self-reported as African American, Asian, Caucasian, or Hispanic on the test requisition form. We curated all reclassifications, reclassifications resulting in downgrades from VUS to benign/likely benign and medically significant reclassifications (MSR), which changed the actionability of a result (i.e., reclassifications from VUS to pathogenic/likely pathogenic and newly detected deep intronic PV).

Results: A total of 39,092 individuals were included in this study. At least one VUS was reported in 40.5% of non-white and Hispanic individuals and in 32.4% of NHW individuals. PV were reported in 11.1% of non-white and Hispanic individuals and in 12.5% of NHW individuals. Use of RNA evidence led to variant reclassifications in 1.7% of non-white and Hispanics compared to 1.1% of NHW ($p<0.001$). Specifically, MSR were made in 1.3% of non-white and Hispanic individuals compared to 0.7% of NHW ($p<0.001$). This increased the positive rate by 2.6% and decreased the VUS rate by 2.2% in non-white and Hispanic individuals. These changes were greater than the increase in PV rate (+1.6%; $p=0.03$) and decrease in VUS rate (-1.4%; $p<0.001$) observed in NHW. These trends were observed when comparing individual racial and ethnic groups, though they were not always statistically significant due to smaller sample size. The notable exception was in Hispanic individuals, where there was no significant difference in any of

the metrics evaluated compared to NHW. Details for specific racial and ethnic groups are provided in table 1.

Conclusion: Our results indicate that RNA sequencing may be of particular benefit to underrepresented populations. RNA evidence had a greater impact on PV and VUS rate in non-white and Hispanic relative to NHW individuals in our cohort. The observed disparities that persisted highlight the need for continued efforts to improve accuracy and utility of genetic testing across racial and ethnic groups; however, wide-scale concurrent availability of RNA evidence can play a role towards improved equity in variant classification.

Table 1. Impact of RNA Evidence by Race/Ethnicity

	Any Reclassification % (number impacted; p-value)	MSR and VUS downgrade % (number impacted; p-value)	Relative Increase in PV Rate % (number impacted; p-value)	Relative Decrease in VUS Rate % (number impacted; p-value)
NHW	1.1% (308; reference)	0.7% (196; reference)	+1.6% (56; reference)	-1.4% (124; reference)
All Non-white and Hispanic	**1.7% (189; p<0.001)**	**1.3% (141; p<0.001)**	**+2.6% (32; p=0.03)**	**-2.2% (101; p<0.001)**
African American	1.4% (44; p=0.11)	**1.4% (42; p<0.001)**	**+3.7% (11; p=0.01)**	**-2.3% (30; p=0.01)**
Asian	**4.3% (66; p<0.001)**	**2.9% (45; p<0.001)**	+2.5% (4; p=0.41)	**-4.4% (38; p<0.001)**
Hispanic	1.2% (30; p=0.58)	0.6% (14; p=0.46)	+1.6% (4; p=0.96)	-1.1% (10; p=0.42)