

THE ROLE OF HIGH THROUGHPUT FUNCTIONAL EVIDENCE IN REDUCING
POPULATION-SPECIFIC DIFFERENCES IN THE QUALITY OF VARIANT
INTERPRETATION

Makenzie Fong, Taylor Silkey

May 2022

Submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Human Genetics
Sarah Lawrence College

Abstract

Variants of uncertain significance (VUSs) in cancer pre-disposition genes are more frequent in non-White and/or Hispanic populations than non-Hispanic White (NHW), creating more ambiguity in cancer risk/management for these populations. High throughput functional evidence (HTFE) serves as a powerful classification tool for missense variants within the RING/BRCT domains of *BRCA1*. We sought to determine if HTFE provides preferential benefit in underrepresented racial and ethnic groups. Our cohort consisted of individuals who had *BRCA1* testing and self-reported ancestry as Asian, Black, Hispanic, Middle Eastern, or NHW. For individuals with pathogenic, likely pathogenic, or VUS variants in the RING/BRCT domains, we evaluated the frequency at which HTFE was applied and at which HTFE impacted variant classification, and compared this between non-White and/or Hispanic individuals and NHW individuals using Fisher's exact test. We found that while application of HTFE between non-White and/or Hispanic individuals (n=140; 64.81%) and NHW individuals (n=240; 69.36%) did not differ, non-White and/or Hispanic individuals (n=67; 31.02%) were more likely to have a variant upgraded due to HTFE than NHW (n=63; 18.21%) (OR=2.0; 95% CI: 1.4-3.0; p<0.001). Based on these preliminary results, HTFE could improve equity in variant assessment. This impact is likely an underestimate, as variants downgraded to likely benign/benign were not evaluated. As part of the larger goal towards genetic testing equity, efforts to expand HTFE availability to additional variants/genes may be warranted.

Acknowledgements

We would like to express sincere gratitude to our mentor, Carrie Hortorn, for guiding us through every step of this project. We learned a lot from Carrie and we appreciate her patience in teaching us along the way. Our advisor, Claire Davis, also played an integral role throughout this process, and we are grateful for her thoughtful comments and feedback. Finally, we would like to extend thanks to Tim Komala, our data analyst at Ambry Genetics, for providing us with quality data and analysis insights. We could not have done this without all of these individuals' support.

Introduction

Accurate identification and classification of pathogenic variants in hereditary cancer predisposition genes allow for risk stratification and personalized management for individuals at high risk to develop cancer. Particularly in Hereditary Breast and Ovarian Cancer (HBOC) syndrome, germline pathogenic variants in related genes can clarify future risk of developing cancer and guide clinicians and patients in curating a personalized screening and risk reduction plan (NCCN HBOPC, 2021). However, individuals from non-White and/or Hispanic populations who pursue multigene panel testing (MGPT) for variants in cancer predisposition genes often receive less informative genetic testing results than those from non-Hispanic White (NHW) backgrounds. Several studies have reported that patients from non-White and/or Hispanic populations receive variants of uncertain significance (VUSs) more often than NHW populations (Caswell-Jin et al., 2018; Kurian, 2018; Ndugga-Kabuye & Issaka, 2019; Roberts et al., 2020). For example, Ndugga-Kabuye and Issaka (2019) reported that VUS frequencies in HBOC and Lynch Syndrome (LS) genes were higher in patients with self-reported Hispanic, African, or Asian/Pacific Islander ancestry than in individuals with self-reported European ancestry (7.1%, 12.3%, 13.1%, vs. 5.8%, respectively). This discrepancy is apparent for genetic test results related to other cancer genes (e.g. *TP53*, *BAP1*, *MITF*, others) as well (16.2%, 21.6%, 24.4%, vs. 12.2%, respectively).

In contrast to the clear utility of identifying a pathogenic variant, it is well established that VUSs complicate risk assessment and medical management through their ambiguity, subsequent misinterpretation, and eventual reclassification (Hoffman-Andrews, 2017). VUSs do not provide concrete information for cancer risk assessment. Insufficient data on functional impact and the fact that most VUSs are reclassified to benign prompt the stance that VUS results

should not be used to guide management recommendations (NCCN HBOPC, 2021). Another concern is misinterpretation of VUSs when VUS results are used to make inappropriate recommendations for screening and risk-reducing surgeries, such as a prophylactic mastectomy (Donohue et al., 2021; Kurian et al., 2017). Reclassification of VUSs presents further challenges. In cases where VUS results are reclassified to likely benign/benign (LB/B), misinterpretation of the initial result introduces unnecessary stress and morbidities associated with high-risk screenings and preventive procedures pursued before reclassification (Macklin, et al., 2018; Slavin et al., 2018, 2019). Similarly, for VUSs that are reclassified as likely pathogenic/pathogenic (LP/P), proper management is delayed and cancer risk is not appropriately managed in the interim.

In addition to preventing personalization of management recommendations, the discussion and disclosure of VUSs during pre-test and post-test genetic counseling sessions create unique counseling dilemmas for healthcare providers and their patients. Describing the possibility and implications of inconclusive results is an integral piece of informed consent, but there are no specific guidelines on how to convey this information, nor on how to disclose a VUS result (Riley et al., 2011). This creates confusion and frustration among providers (Medendrop et al., 2018). Providers both within and outside of genetics specialties have expressed that VUS results disclosure is one of the most difficult aspects of counseling (Macklin, et al., 2018; Scherr et al., 2015; Vears, et al., 2019). From the patient perspective, studies have shown that patients who receive a VUS result are at risk of feeling discomfort, frustration, regret, anxiety, or similar negative emotions at the time of disclosure due to the result's unique ambiguity (Clift et al., 2019; Lumish et al., 2017; Macklin, et al., 2018). For patients with VUSs in cancer predisposition genes, a common stressor is the uncertainty of whether or not cancer risk is being

managed appropriately (Makhnoon, et al., 2019; Soloman et al., 2017). Additionally, patients are more likely to misinterpret these results, even when the provider accurately relays the information (Makhnoon, et al., 2019; Richter et al., 2013). Individuals with VUSs may view their result more as pathogenic and consider high-risk screening and/or risk-reducing measures despite counseling (Lumish et al., 2017). This, again, could result in unnecessary procedures.

Because VUSs are more frequently reported for individuals from non-White and/or Hispanic populations, the confusion and burdens associated with VUSs disproportionately affect these groups. This inequity is in part the consequence of variant classification methods that rely on a large volume of data. Population databases used in variant classification do not represent all races and ethnicities equally. For example, one of the databases, gnomAD, is composed of 45% non-Finnish European individuals and only 8.67% African American individuals (Karczewski & Francioli, 2017). Skewed proportions in databases used for variant classification is exacerbated further by lower rates of testing uptake in non-White and/or Hispanic populations (Carroll et al., 2019). Lack of representation in clinical testing cohorts, published literature, and population reference databases creates gaps in evidence available for variant interpretation. Evidence lines can be clinically driven (i.e. co-segregation, de novo observation), based on frequency calculations (i.e. case-control studies), predictive (i.e. *in silico* modeling), or derived from functional data (i.e. *in vitro* assays) (Mester & Pesaran, 2020). Aside from computational approaches, many of these classification tools and criteria rely on volume of observations. Without this volume, interpretation of variants detected in underrepresented populations is limited.

The use of a new variant interpretation technique, coined Multiplexed Assays for Variant Effect (MAVEs), provides the potential to overcome some of the problems attributed to the

volume of observations. Functional study data can be used as strong evidence to inform variant classification (Richards et al., 2015). Traditionally, functional assays are built for each variant after it is detected in high-risk individuals ascertained through clinical or research genetic testing, which is a slow and expensive process (Starita et al., 2017). In contrast, newly developed MAVE approaches to functional interrogation, including high-throughput functional assays, deep mutational scanning, and massively parallel reporter assays, can assess a large number of variants in a single experiment (Starita et al., 2017). This means that every possible missense variant can be evaluated without needing to first be observed. The functional scores derived from MAVEs can be used as high throughput functional evidence (HTFE) during variant classification. While the original intention was to utilize HTFE data to keep pace with increased variant detection due to adoption of multigene panel testing, MAVEs may have particular benefit of filling evidence gaps that affect non-White and/or Hispanic individuals by improving accuracy of variant classification and potentially lowering the frequencies of VUS results in non-White and/or Hispanic populations.

Currently, HTFE has been validated for application to missense variants in the RING/BRCT domain of *BRCA1* (Findlay, 2018; Starita et al., 2015) using the ACMG framework for functional evidence (Brnich, 2020). Prior research has shown that MAVEs can be used to improve reclassification of *BRCA1* VUSs within this domain (Kim et al., 2020). However, the impact of MAVEs has not been compared across racial and ethnic groups. In this study, we aim to explore how MAVE data influences VUS reclassification of *BRCA1* variants in non-White and/or Hispanic populations. We predict that the addition of HTFE will improve VUS reclassification rates preferentially in non-White and/or Hispanic populations.

Methods

Study Population

We retrospectively reviewed de-identified test results, associated demographics, and testing indication for individuals undergoing MGPT for hereditary cancer predisposition at a single diagnostic testing laboratory (Ambry Genetics) between January 2016 through September 2020. Inclusion criteria consisted of the presence of *BRCAl* within the MGPT order (1 gene minimum, 91 genes maximum) and at least one *BRCAl* variant classified as LP/P or VUS. Self-reported race/ethnicity categories were as follows: Asian, Ashkenazi Jewish, Black, Hispanic, Middle Eastern, Native American, NHW. Self-reported Multiracial, Other, or Unknown races/ethnicities were excluded from the study due to ambiguity within these groups that could confound the analysis. Races and ethnicities included in Other are listed in Supplemental Table 1. This study was reviewed by the Sarah Lawrence College Institutional Review Board and was determined to be exempt.

Testing and Results Interpretation

Next generation sequencing and deletion duplication testing was performed as previously described (Mu et al., 2019). Briefly, genomic DNA was isolated from a blood, saliva, or fibroblast sample and then quantified (Nanodrop technology provided by Thermo Scientific based in Pittsburgh, Pennsylvania; or Infinite F200 technology provided by Tecan, based in San Jose, California). Sequence enrichment was performed by incorporating genomic DNA onto a microfluidics chip or into microdroplets with primer pairs or by a bait-capture methodology using long biotinylated oligonucleotide probes (RainDance Technologies, Billerica, Massachusetts; or Integrated DNA Technologies, San Diego, California). This procedure was then followed by next-generation sequencing analysis (Illumina, San Diego, California) of all

coding exons in addition to at least five bases into the 5' and 3' ends of all introns. Sanger sequencing was performed to confirm variant calls in regions missing or with insufficient read depth coverage, variants in regions complicated by pseudogene interference, and potentially homozygous variants. Gross deletion/duplication analysis for all genes was also performed using a custom pipeline based on read-depth from NGS data and/or targeted chromosomal microarray with confirmatory MLPA when applicable (Mu et al., 2019).

Test results including overall report classification (positive, negative, inconclusive) and individual variant classification were detailed. Interpretation of sequence variations was performed according to the American College of Medical Genetics and Genomics guidelines (Richards, 2015). Variants identified by MGPT were classified as pathogenic (P), likely pathogenic (LP), variant of unknown significance (VUS), likely benign (LB), or benign (B) according to the Ambry five-tier variant classification protocol (Pesaran et al., 2016). Cases with at least one pathogenic or likely pathogenic variant, with or without VUS, were defined as positive results. Cases with VUS in the absence of a LP/P variant were defined as inconclusive. Cases with LB/B findings in the absence of a LP/P or VUS findings were defined as negative and were excluded from further analysis.

Application of Functional Evidence and Impact on Classification

Lines of evidence used towards classification of missense variants were collated. Specifically, with regards to functional data, we assessed whether a variant was found to have deleterious function via high-throughput functional assay(s), deleterious function via other functional assay, intact function via high-throughput functional assay, intact function via other functional assay, or conflicting functional evidence. Variants falling within the RING domain (nucleotides 1-301) and the BRCT domain (nucleotides 4950-5589) were specifically evaluated

because high-throughput functional assays applicable to *BRCA1* variants in the RING/BRCT domain have been described previously (Findlay, 2018; Starita et al., 2015).

We compared how often HTFE was applied towards classification of missense variants in the RING/BRCT domain between NHW and racial and ethnic groups that historically have been underrepresented in genetic testing cohorts (Asian, Black, Hispanic, and Middle Eastern).

Furthermore, we compared how often HTFE applied to variants in the RING/BRCT domain led to medically significant reclassifications (MSR), defined as reclassifications that changed the actionability of a result (i.e., VUS to LP/P) between NHW and underrepresented cohorts. A Fisher's exact test was used to determine statistical significance in both comparisons.

Results

Cohort Demographics

358,721 individuals had testing including *BRCA1* during our study period. We identified 8,171 reportable variants (LP/P and VUS) in 8,036 persons (2.24% of all those tested) during the study period. Our cohort was predominantly female (88.35%) with an average age of 49.76 at the time of testing. 71.29% had a personal history of cancer and 28.71% had no personal history of cancer. 11.73% of patients identified as Black, 11.01% as Ashkenazi Jewish, 8.09% as Asian, 10.42% as Hispanic, 1.03% as Middle Eastern, 0.04% as Native American, and 57.68% as non-Hispanic White.

Table 1.*Cohort Demographics*

	All	Black	AJ	Asian	Hispanic	Middle Eastern	NA	NHW
Total Tested	358721 ^a	27526	19621	15568	25550	2154	407	217050
Total with Reportable BRCA1	8036	943	885	650	837	83	3	4635
Age								
Mean	49.76	48.45	48.51	48.12	47.08	46.96	45.33	51.04
Median	50	48	48	47	46	45	41	51
Cancer History								
Affected								
N	5729	742	470	512	630	47	3	3325
% Total Tested	1.60%	2.70%	2.40%	3.29%	2.47%	2.18%	0.74%	1.53%
% Study Cohort	71.29%	78.69%	53.11%	78.77%	75.27%	56.63%	100.00%	71.74%
Unaffected								
N	2307	201	415	138	207	36	0	1310
% Total Tested	0.64%	0.73%	2.12%	0.89%	0.81%	1.67%	0.00%	0.60%
% Study Cohort	28.71%	21.31%	46.89%	21.23%	24.73%	43.37%	0.00%	28.26%
Sex								
Female								
N	7100	893	644	604	786	74	3	4096
% Total Tested	1.98%	3.24%	3.28%	3.88%	3.08%	3.44%	0.74%	1.89%
% Study Cohort	88.35%	94.70%	72.77%	92.92%	93.91%	89.16%	100.00%	88.37%
Male								

N	932	50	240	46	51	9	0	536
% Total Tested	0.26%	0.18%	1.22%	0.30%	0.20%	0.42%	0.00%	0.25%
% Study Cohort	11.60%	5.30%	27.12%	7.08%	6.09%	10.84%	0.00%	11.56%
Not Reported								
N	4	0	1	0	0	0	0	3
% Total Tested	0.00%	0.00%	0.01%	0.00%	0.00%	0.00%	0.00%	0.00%
% Study Cohort	0.05%	0.00%	0.11%	0.00%	0.00%	0.00%	0.00%	0.06%

Note. AJ = Ashkenazi Jewish, NA = Native American

^aThis number represents all individuals tested during our study period, including those that did not self-report race/ethnicity as Black, Ashkenazi Jewish, Asian, Hispanic, Middle Eastern, and NHW.

***BRCA1* Results**

Of the 358,721 total individuals tested, 5,072 (1.41%) had a pathogenic variant in *BRCA1*, 185 (0.05%) had a likely pathogenic variant in *BRCA1*, and 2,915 (0.81%) had a VUS in *BRCA1*, including 158 individuals with more than one reportable *BRCA1* variant. A positive (pathogenic or likely pathogenic) result was seen most frequently in Hispanic individuals (2.28%) followed by Middle Eastern individuals (2.14%), Asian individuals (2.01%), Black individuals (1.85%), and NHW individuals (1.38%). An inconclusive result (VUS) was seen most frequently in Asian individuals (2.29%) followed by Middle Eastern individuals (1.72%), Black individuals (1.65%), Hispanic individuals (1.03%), and NHW individuals, (0.79%). Native American individuals were excluded from analysis due to the small number of reportable *BRCA1* variants (n=3). Additionally, while Ashkenazi Jewish ancestry differs from NHW, and Ashkenazi

Jewish populations are known as a marginalized population in a socio-political sense, they are not underrepresented in genetic databases and were excluded from further analysis. Rates of positive and inconclusive results were higher in each underrepresented racial and ethnic group studied compared to NHW ($p < 0.001$ for each comparison).

Table 2.

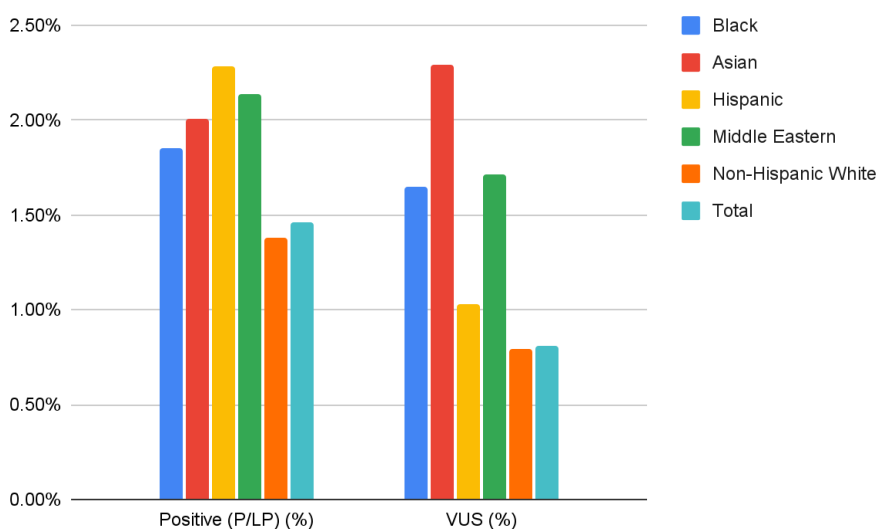
BRCA1 Results by Race and Ethnicity (% Total Tested)

	Total	Black	Asian	Hispanic	Middle Eastern	NHW
Pathogenic	5072 ^a (1.41%)	480 (1.74%)	284 (1.82%)	567 (2.22%)	43 (2.00%)	2890 (1.33%)
Likely Pathogenic	185 ^a (0.05%)	29 (0.11%)	29 (0.19%)	16 (0.06%)	3 (0.14%)	103 (0.05%)
VUS	2915 ^a (0.81%)	454 (1.65%)	357 (2.29%)	264 (1.03%)	37 (1.72%)	1723 (0.79%)

^aThese numbers include Ashkenazi Jewish and Native American individuals.

Figure 1.

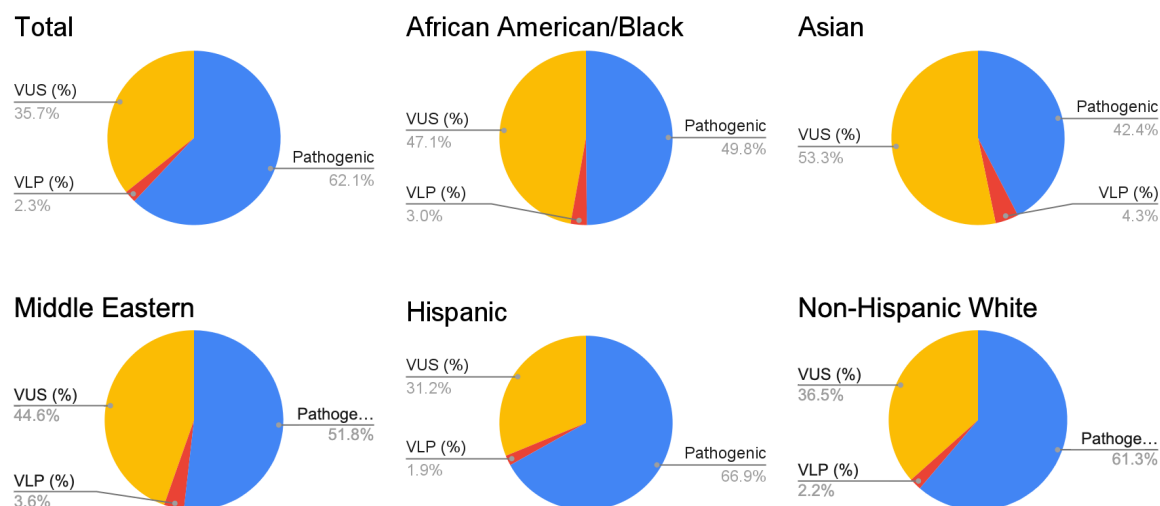
BRCA1 Results by Race and Ethnicity (% Total Tested)



Note. Total includes Ashkenazi Jewish and Native American individuals.

Figure 2.

BRCA1 Results by Race and Ethnicity (% Individuals with Reportable BRCA1 Variant)



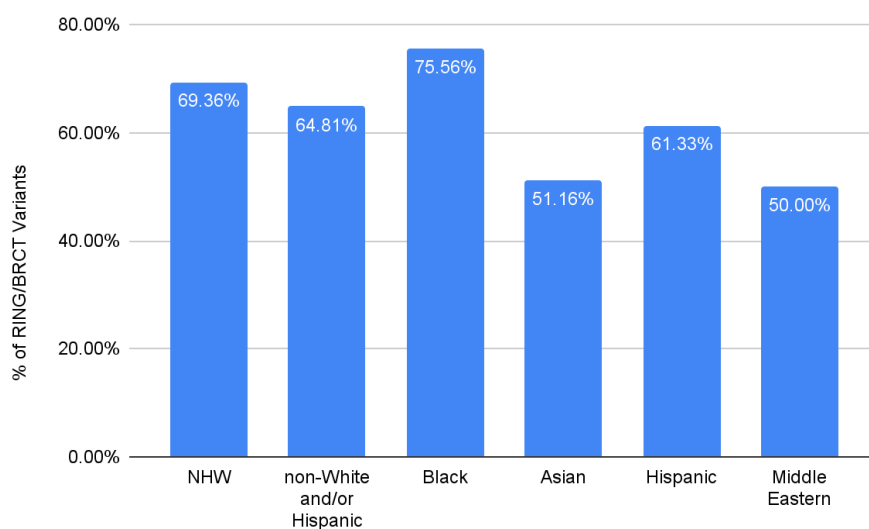
HTFE Application and Impact

To examine whether an inherent bias existed in which domain missense variants were observed more often in NHW individuals, we assessed the frequency of domain missense variants in each group and found that domain missense variants were observed more frequently in non-White and/or Hispanic individuals than NHW ($p < 0.001$). See Table 2 in Supplement.

Out of 8,171 reportable variants, 3,074 (37.62%) were missense variants. Of these, 562 (6.88%) were missense variants located within the RING/BRCT domains that had HTFE available. While only a small minority of individuals in our cohort had variants included in the RING/BRCT domain, this evidence type was applied frequently in eligible variants (Table 2). Overall, HTFE was applied in 380 (67.62%) individuals with missense variants found in the RING/BRCT domain. We did not find a statistically significant difference in the application of HTFE between non-White and/or Hispanic individuals ($n = 140$; 64.81%) and NHW individuals ($n = 240$; 69.36%).

Table 3.*Application of HTFE by Race and Ethnicity (% of RING/BRCT variants)*

	Black	Asian	Hispanic	Middle Eastern	non-White and/or Hispanic	NHW
Pathogenic	48 (53.33%)	10 (23.26%)	32 (42.67%)	0 (0.00%)	90 (41.67%)	176 (50.87%)
Likely Pathogenic	14 (15.56%)	8 (18.60%)	10 (13.33%)	0 (0.00%)	32 (14.81%)	34 (9.83%)
VUS	6 (6.67%)	4 (9.30%)	4 (5.33%)	4 (50.00%)	18 (8.33%)	30 (8.67%)
Any Reportable	68 (75.56%)	22 (51.16%)	46 (61.33%)	4 (50.00%)	140 (64.81%)	240 (69.36%)

Figure 3.*Application of HTFE by Race and Ethnicity*

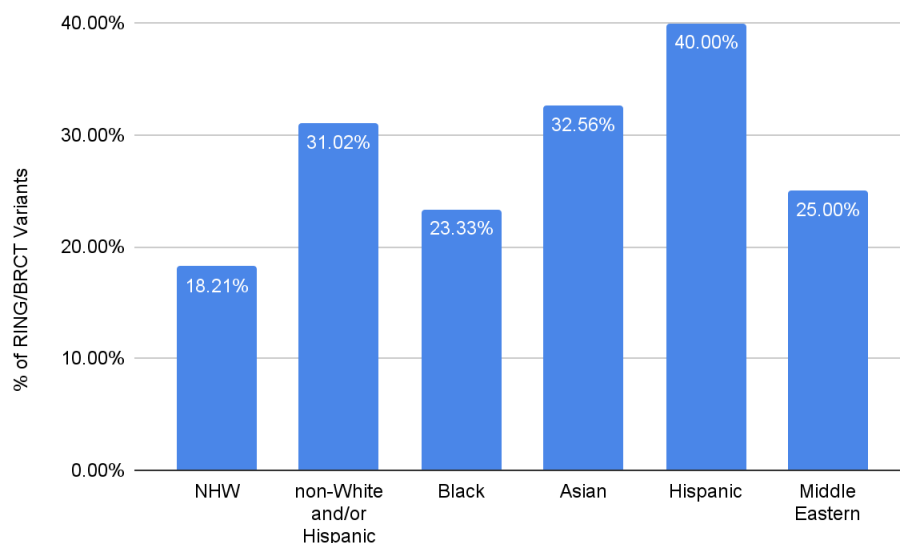
When HTFE was applied, a reclassification upgrade (VUS to LP or LP to P) was made in 34.21% (n=130) of individuals. Notably, 105 of the upgrades were VUS to LP, representing a change in actionability due to eligibility for increased surveillance, surgical, and/or treatment

options. Non-White and/or Hispanic individuals (n = 67; 31.02%) were more likely to have a variant upgraded due to HTFE compared to NHW (n = 63; 18.21%) (OR = 2.0; 95% CI: 1.4-3.0; $p < 0.001$). When we looked at individual groups, we found that HTFE was more likely to impact classification in Hispanic individuals (n = 30; 40.00%) (OR = 3.0; 95% CI: 1.8-5.1; $p < 0.001$), and in Asian individuals (n = 14; 32.56%) (OR = 2.2; 95% CI: 1.1-4.3; $p = 0.03$) than in NHW individuals. While the frequency at which HTFE led to a classification upgrade was still higher in individuals who identified as Black and Middle Eastern, this did not reach statistical significance, possibly due to lack of power when racial/ethnic groups were stratified.

Table 4.

HTFE Impacted Classification (% of RING/BRCT variants)

	Black	Asian	Hispanic	Middle Eastern	non-White and/or Hispanic	NHW
Pathogenic	0 (0.00%)	2 (4.65%)	18 (24.00%)	0 (0.00%)	20 (9.26%)	8 (2.31%)
Likely Pathogenic	10 (11.11%)	1 (2.33%)	1 (1.33%)	0 (0.00%)	12 (5.56%)	7 (2.02%)
VUS	11 (12.22%)	11 (25.58%)	11 (14.67%)	2 (25.00%)	35 (16.20%)	48 (13.87%)
Any Reportable	21 (23.33%)	14 (32.56%)	30 (40.00%)	2 (25.00%)	67 (31.02%)	63 (18.21%)

Figure 4.*HTFE Impact on Classification by Race and Ethnicity***Discussion**

We assessed the application and impact of HTFE on variant reclassification across racial and ethnic groups to determine if HTFE improves variant classification in non-White and/or Hispanic groups historically underrepresented in genetic testing databases. We found that while there was no difference in the application of HTFE between non-White and/or Hispanic individuals and NHW individuals, HTFE preferentially improved variant interpretation in non-White and/or Hispanic individuals. These findings indicate that this novel source of evidence may ameliorate discrepancy in the clinical utility of genetic testing between NHW and non-White and/or Hispanic individuals, as all individuals of races and/or ethnicities other than NHW received upgrades more frequently than NHW individuals. Larger sample sizes may be required to determine statistical significance in some racial and ethnic groups (i.e. among Black and Middle Eastern individuals).

In our cohort of patients with reportable *BRCA1* variants, we confirmed the findings of previous studies that non-White and/or Hispanic individuals receive VUSs at a higher frequency than NHW individuals (Caswell-Jin et al., 2018; Jones et al., 2019). We also compared rates of pathogenic and likely pathogenic classification of variants across groups and found that pathogenic variants were found in a greater proportion of patients from non-White and/or Hispanic individuals than NHW individuals. This is consistent with trends observed in a previous cohort tested at Ambry Genetics (Yadav, 2020). An explanation for this could be that non-White and/or Hispanic individuals are more likely to be personally affected with cancer before a referral for genetic testing is made, indicating they may have a higher *a priori* risk of a pathogenic variant.

This study addresses the urgent need to improve risk assessment and personalized management among non-White and/or Hispanic individuals. Most notably, the results of this study indicate that HTFE may improve variant classification among non-White and/or Hispanic individuals. This would facilitate more accurate risk assessment and appropriate risk-reducing medical management, especially when testing targets actionable genes with published management guidelines, such as HBOC genes, as clinicians report making changes to cancer risk recommendations in three-quarters of patients with positive results (Horton et al., 2022). Ultimately, this could lead to large advancements in preventative medicine, both within and outside of oncology.

Future Recommendations

This study analyzed early efforts to assess the utility of MAVEs in underrepresented populations. Although we found that HTFE can improve classification upgrade in non-White and/or Hispanic individuals with variants in the BRCT/RING domain, there are other missense

variants in *BRCA1* outside of the RING/BRCT domain where HTFE is not available. We observed that while HTFE led to upgrades in 34.21% (n=130) of *BRCA1* domain missense variants where HTFE was applied, these variants represent a small minority of VUSs identified via MGPT. In order to maximize the benefit of this powerful tool, efforts must be made to increase the number of HTFE validated for clinical evidence in other domains of *BRCA1* and in other genes. Additionally, proactive reassessment of rarely observed variants in the context of HTFE could also improve the quality of care for non-White and/or Hispanic patients in oncology and other areas of medicine.

A dataset including all LB/Bs would better reflect the full impact of HTFE on variant reclassification. Within our dataset of individuals with reportable *BRCA1* variants, we did identify 263 patients who also carried a LB variant in *BRCA1*. We observed a similar trend in downgrades, in which 67% of LBs were dependent on HTFE in non-White and/or Hispanic individuals compared to 52.6% of LB variants in NHW individuals. However, the significance of this is limited because we were only able to assess variants in individuals who also had a reportable *BRCA1* variant. A majority of VUSs are downgraded to LB/B rather than upgraded to LP/P once reclassified (Mersch et al., 2018). Therefore, we can hypothesize that if a VUS is to be reclassified by HTFE, the probable reclassification is to LB/B. Within the timeframe of this study, we were unable to comb through the downgrade data and ascertain how many VUSs were downgraded after the application of HTFE within each racial and ethnic group. To fully assess the utility of HTFE in non-White and/or Hispanic individuals, frequencies of downgrade from VUS to LB/B should be calculated for all racial and ethnic groups. While downgrades do not change clinical management, they are influential in easing anxiety and limiting misinterpretation by patients and their providers, ultimately improving care, clarity, and safety.

This study sought to address one of many sources of disparity in genetic testing; however, there are more factors that contribute to differential outcomes across racial and ethnic groups. For example, our study cohort remains subject to referral bias, as over half of our participants identified as NHW, sample sizes for Black and Middle Eastern may have been too small to be considered statistically significant, and our sample size for Native American was so small that we could not include the population in the analysis.

Lower genetic testing rates in non-White and/or Hispanic populations is a vast and complex problem with several contributing socio-political factors. Often, access to genetic testing begins with a discussion between provider and patient and a referral to genetic counseling. Early studies found that Black women with a family history of breast or ovarian cancer were less likely to have genetic counseling for *BRCA1/2* testing than NHW women with a similar family history (Armstrong et al., 2005). This trend has persisted more recently, as several studies found that providers are less likely to refer non-White and/or Hispanic patients to genetic counselors in comparison to NHW patients, even when they meet clinical guidelines for genetic testing (Chapman-Davis et al., 2021; Cragun et al., 2019; Peterson et al., 2020). It is probable that these discrepancies in rates of referral to genetic counseling are partly due to implicit biases of providers and likely contribute to the lower overall awareness of genetic testing among non-White and/or Hispanic patients (Cragun, et al., 2019; FitzGerald & Hurst, 2017; Hann et al., 2017, Schaa et al., 2015).

These biases are just one consequence of the larger and more complex phenomenon of systemic racism, which exacerbates other barriers along the path to receiving genetic testing. Systemic racism is defined as access differences and difficulties among non-White and/or Hispanic populations in comparison to NHW in healthcare, education, housing, employment, and

other opportunities of society, rooted in both historic and current laws, practices, and customs (Elias & Paradies, 2021; Jones, 2000). This type of racism contributes to a wide variety of barriers that some non-White and/or Hispanic individuals have identified as insurance coverage, language barriers, cultural congruence, competing demands (i.e. finding childcare, taking time off work), and general mistrust of healthcare providers and the healthcare system (George, et al., 2014). George, et al. (2014) found that mistrust, especially, was a frequently shared barrier among individuals who identified as Black, Asian, Latinx, or Pacific Islander. The foundation of this hesitancy is the historic and present racism and discrimination of non-White and/or Hispanic individuals in clinical and research settings, with specific examples including, but not limited to, the Tuskegee Syphilis Study, Havasupai Tribe research study, and racially-biased and outdated diagnostic algorithms that change clinical guidelines based on a patient's race or ethnicity (Vyas, et al., 2020).

This is not an exhaustive summary of all of the barriers that non-White and/or Hispanic individuals may face when seeking genetic testing and counseling. The barriers illustrated here are only some of the patterns assessed by researchers. Opinions and perspectives vary significantly within racial, ethnic, and cultural groups, and we are unable to make generalizations about the weight of these concerns within their respective population. Overall we would like to emphasize that, while the findings of our study hold great utility in improving the quality of care of non-White and/or Hispanic patients, we would also like to acknowledge the importance of researching the socio-political heavy barriers to equitable genetic testing in conjunction with improved laboratory techniques.

Limitations

Our study has several limitations. As stated previously, our study cohort remains subject to referral bias. Additionally, we only included individuals who had testing done at Ambry Genetics, so we were not able to determine reproducibility of variant classification impact across genetic testing laboratories. Race and ethnicity was self reported, and was not confirmed via ancestrally informative markers. Within our analyses, we did not assess the application and impact of HTFE in all individuals with an LB/B variant, so we were not able to determine if there are population differences in the impact of HTFE on downgrading variants.

Conclusion

MGPT for HBOC genes can serve as a powerful source of information to guide clinical management for individuals with a personal and/or family history of cancer. However, VUS results limit the clinical utility of this genetic testing. Increased frequencies of VUS results in non-White and/or Hispanic individuals undergoing genetic testing for HBOC genes expands the already large gap in quality of care between NHW and non-White and/or Hispanic individuals (Bentley et al., 2017). Our study suggests that HTFE could reduce existing inequities in classification of *BRCA1* variants among racial and ethnic groups. While HTFE was found to be beneficial for the reclassification of VUSs in all racial and ethnic backgrounds, it could be especially impactful for non-White and/or Hispanic individuals. Therefore, expansion of HTFE availability to additional variants and genes may warrant special attention in efforts to improve equity in variant classification. It is important to note that the paucity of actionable results in non-White and/or Hispanic individuals is only one component of a larger systematic issue of inequitable healthcare. Research should be conducted within a laboratory setting, such as our

study, but also in a socio-political lens, further exploring other barriers that non-White and/or Hispanic patients encounter when receiving healthcare.

References

- Armstrong, K. (2005). Racial differences in the use of BRCA1/2 testing among women with a family history of breast or ovarian cancer. *JAMA*, 293(14), 1729.
<https://doi.org/10.1001/jama.293.14.1729>
- Bentley, A. R., Callier, S., & Rotimi, C. N. (2017). Diversity and inclusion in genomic research: Why The uneven progress? *Journal of Community Genetics*, 8(4), 255–266.
<https://doi.org/10.1007/s12687-017-0316-6>
- Brnich, S. E., Abou Tayoun, A. N., Couch, F. J., Cutting, G. R., Greenblatt, M. S., Heinen, C. D., Kanavy, D. M., Luo, X., McNulty, S. M., Starita, L. M., Tavgian, S. V., Wright, M. W., Harrison, S. M., Biesecker, L. G., & Berg, J. S. (2019). Recommendations for application of the functional evidence PS3/BS3 criterion using the ACMG/AMP sequence variant interpretation framework. *Genome Medicine*, 12(1).
<https://doi.org/10.1186/s13073-019-0690-2>
- Brothers, K. B., Bennett, R. L., & Cho, M. K. (2021). Taking an antiracist posture in scientific publications in human genetics and Genomics. *Genetics in Medicine*, 23(6), 1004–1007.
<https://doi.org/10.1038/s41436-021-01109-w>
- Carroll, N. M., Blum-Barnett, E., Madrid, S. D., Jonas, C., Janes, K., Alvarado, M., Bedoy, R., Paolino, V., Aziz, N., McGlynn, E. A., & Burnett-Hartman, A. N. (2019). Demographic

- differences in the utilization of clinical and direct-to-consumer genetic testing. *Journal of Genetic Counseling*, 29(4), 634–643. <https://doi.org/10.1002/jgc4.1193>
- Caswell-Jin, J. L., Gupta, T., Hall, E., Petrovchich, I. M., Mills, M. A., Kingham, K. E., Koff, R., Chun, N. M., Levonian, P., Lebensohn, A. P., Ford, J. M., & Kurian, A. W. (2018). Racial/ethnic differences in multiple-gene sequencing results for hereditary cancer risk. *Genetics in Medicine*, 20(2), 234–239. <https://doi.org/10.1038/gim.2017.96>
- Clift, K., Macklin, S., Halverson, C., McCormick, J. B., Abu Dabrh, A. M., & Hines, S. (2019). Patients' views on variants of uncertain significance across indications. *Journal of Community Genetics*, 11(2), 139–145. <https://doi.org/10.1007/s12687-019-00434-7>
- Cragun, D., Weidner, A., Kechik, J., & Pal, T. (2019). Genetic testing across young Hispanic and Non-Hispanic white breast cancer survivors: Facilitators, barriers, and awareness of the genetic information nondiscrimination act. *Genetic Testing and Molecular Biomarkers*, 23(2), 75–83. <https://doi.org/10.1089/gtmb.2018.0253>
- Donohue, K. E., Gooch, C., Katz, A., Wakelee, J., Slavotinek, A., & Korf, B. R. (2021). Pitfalls and challenges in genetic test interpretation: An exploration of genetic professionals experience with interpretation of results. *Clinical Genetics*, 99(5), 638–649. <https://doi.org/10.1111/cge.13917>
- Elias, A., & Paradies, Y. (2021). The costs of institutional racism and its Ethical Implications for Healthcare. *Journal of Bioethical Inquiry*, 18(1), 45–58. <https://doi.org/10.1007/s11673-020-10073-0>

- Findlay, G. M., Daza, R. M., Martin, B., Zhang, M. D., Leith, A. P., Gasperini, M., Janizek, J. D., Huang, X., Starita, L. M., & Shendure, J. (2018). Accurate classification of BRCA1 variants with saturation genome editing. *Nature*, *562*(7726), 217–222.
<https://doi.org/10.1038/s41586-018-0461-z>
- FitzGerald, C., & Hurst, S. (2017). Implicit bias in healthcare professionals: A systematic review. *BMC Medical Ethics*, *18*(1). <https://doi.org/10.1186/s12910-017-0179-8>
- George, S., Duran, N., & Norris, K. (2014). A systematic review of barriers and facilitators to minority research participation among African Americans, Latinos, Asian Americans, and Pacific Islanders. *American Journal of Public Health*, *104*(2).
<https://doi.org/10.2105/ajph.2013.301706>
- Hann, K. E., Freeman, M., Fraser, L., Waller, J., Sanderson, S. C., Rahman, B., Side, L., Gessler, S., & Lanceley, A. (2017). Awareness, knowledge, perceptions, and attitudes towards genetic testing for cancer risk among ethnic minority groups: A systematic review. *BMC Public Health*, *17*(1). <https://doi.org/10.1186/s12889-017-4375-8>
- Hoffman-Andrews, L. (2017). The known unknown: The challenges of genetic variants of uncertain significance in clinical practice. *Journal of Law and the Biosciences*, *4*(3), 648–657. <https://doi.org/10.1093/jlb/lxx038>
- Horton, C., Blanco, K., Lo, M.-T., Speare, V., LaDuca, H., Dolinsky, J. S., & Kurian, A. W. (2022). Clinician-Reported Impact of Germline Multigene Panel Testing on Cancer Risk Management Recommendations. *JNCI Cancer Spectrum*, *6*(2), pkac002.
<https://doi.org/10.1093/jncics/pkac002>

- Jones, C. P. (2000). Levels of racism: A theoretic framework and a gardener's tale. *American Journal of Public Health*, 90(8), 1212–1215. <https://doi.org/10.2105/ajph.90.8.1212>
- Jones, T., Trivedi, M. S., Jiang, X., Silverman, T., Underhill, M., Chung, W. K., Kukafka, R., & Crew, K. D. (2019). Racial and ethnic differences in BRCA1/2 and Multigene panel testing among Young Breast Cancer Patients. *Journal of Cancer Education*, 36(3), 463–469. <https://doi.org/10.1007/s13187-019-01646-8>
- Karczewski, K., & Francioli, L. (2017, February 27). The genome Aggregation Database (gnomAD) | gnomAD news. GnomAD News. <https://gnomad.broadinstitute.org/news/2017-02-the-genome-aggregation-database/>
- Kim, H.-K., Lee, E. J., Lee, Y.-J., Kim, J., Kim, Y., Kim, K., Lee, S.-W., Chang, S., Lee, Y. J., Lee, J. W., Lee, W., Chun, S., Son, B. H., Jung, K. H., Kim, Y.-M., Min, W.-K., & Ahn, S.-H. (2020). Impact of proactive high-throughput functional assay data on BRCA1 variant interpretation in 3684 patients with breast or ovarian cancer. *Journal of Human Genetics*, 65(3), 209–220. <https://doi.org/10.1038/s10038-019-0713-2>
- Kurian, A. W., Li, Y., Hamilton, A. S., Ward, K. C., Hawley, S. T., Morrow, M., McLeod, M. C., Jagsi, R., & Katz, S. J. (2017). Gaps in incorporating germline genetic testing into treatment decision-making for early-stage breast cancer. *Journal of Clinical Oncology*, 35(20), 2232–2239. <https://doi.org/10.1200/jco.2016.71.6480>
- Kurian, A. W., Ward, K. C., Hamilton, A. S., Deapen, D. M., Abrahamse, P., Bondarenko, I., Li, Y., Hawley, S. T., Morrow, M., Jagsi, R., & Katz, S. J. (2018). Uptake, results, and

outcomes of germline multiple-gene sequencing after diagnosis of breast cancer. *JAMA Oncology*, 4(8), 1066. <https://doi.org/10.1001/jamaoncol.2018.0644>

Lumish, H. S., Steinfeld, H., Koval, C., Russo, D., Levinson, E., Wynn, J., Duong, J., & Chung, W. K. (2017). Impact of panel gene testing for hereditary breast and ovarian cancer on patients. *Journal of Genetic Counseling*, 26(5), 1116–1129.

<https://doi.org/10.1007/s10897-017-0090-y>

Macklin, S. K., Jackson, J. L., Atwal, P. S., & Hines, S. L. (2018). Physician interpretation of variants of uncertain significance. *Familial Cancer*, 18(1), 121–126.

<https://doi.org/10.1007/s10689-018-0086-2>

Makhnoon, S., Shirts, B. H., & Bowen, D. J. (2019). Patients' perspectives of variants of uncertain significance and strategies for uncertainty management. *Journal of Genetic Counseling*, 28(2), 313–325. <https://doi.org/10.1002/jgc4.1075>

Medendorp, N. M., Hillen, M. A., Murugesu, L., Aalfs, C. M., Stiggelbout, A. M., & Smets, E. M. (2018). Uncertainty related to Multigene panel testing for cancer: A qualitative study on counsellors' and counselees' views. *Journal of Community Genetics*, 10(2), 303–312.

<https://doi.org/10.1007/s12687-018-0393-1>

Mester, J., & Pesaran, T. (2020). The evolution of constitutional sequence variant interpretation. *Clinics in Laboratory Medicine*, 40(2), 135–148.

<https://doi.org/10.1016/j.cll.2020.02.005>

Mu, W., Li, B., Wu, S., Chen, J., Sain, D., Xu, D., Black, M. H., Karam, R., Gillespie, K., Farwell Hagman, K. D., Guidugli, L., Pronold, M., Elliott, A., & Lu, H.-M. (2019).

Detection of structural variation using target captured next-generation sequencing data for genetic diagnostic testing. *Genetics in Medicine*, 21(7), 1603–1610.

<https://doi.org/10.1038/s41436-018-0397-6>

Ndugga-Kabuye, M. K., & Issaka, R. B. (2019). Inequities in multi-gene hereditary cancer testing: Lower Diagnostic Yield and higher vus rate in individuals who identify as Hispanic, African or Asian and Pacific Islander as compared to European. *Familial Cancer*, 18(4), 465–469. <https://doi.org/10.1007/s10689-019-00144-6>

Pesaran, T., Karam, R., Huether, R., Li, S., Farber-Katz, S., Chamberlin, A., Chong, H., LaDuca, H., & Elliott, A. (2016). Beyond DNA: An Integrated and Functional Approach for Classifying Germline Variants in Breast Cancer Genes. *International Journal of Breast Cancer*, 2016, 2469523. <https://doi.org/10.1155/2016/2469523>

Richards, S., Aziz, N., Bale, S., Bick, D., Das, S., Gastier-Foster, J., Grody, W. W., Hegde, M., Lyon, E., Spector, E., Voelkerding, K., & Rehm, H. L. (2015). Standards and guidelines for the interpretation of sequence variants: A joint consensus recommendation of the American College of Medical Genetics and genomics and the Association for Molecular Pathology. *Genetics in Medicine*, 17(5), 405–424. <https://doi.org/10.1038/gim.2015.30>

Richter, S., Haroun, I., Graham, T. C., Eisen, A., Kiss, A., & Warner, E. (2013). Variants of unknown significance in BRCA testing: Impact on risk perception, worry, prevention and counseling. *Annals of Oncology*, 24, viii69–viii74.

<https://doi.org/10.1093/annonc/mdt312>

Riley, B. D., Culver, J. O., Skrzynia, C., Senter, L. A., Peters, J. A., Costalas, J. W., Callif-Daley, F., Grumet, S. C., Hunt, K. S., Nagy, R. S., McKinnon, W. C., Petrucelli, N. M., Bennett, R. L., & Trepanier, A. M. (2011). Essential elements of genetic cancer risk assessment, counseling, and testing: Updated recommendations of the National Society of Genetic counselors. *Journal of Genetic Counseling*, *21*(2), 151–161.

<https://doi.org/10.1007/s10897-011-9462-x>

Roberts, M. E., Susswein, L. R., Janice Cheng, W., Carter, N. J., Carter, A. C., Klein, R. T., Hruska, K. S., & Marshall, M. L. (2020). Ancestry-specific hereditary cancer panel yields: Moving toward more personalized risk assessment. *Journal of Genetic Counseling*, *29*(4), 598–606. <https://doi.org/10.1002/jgc4.1257>

Schaa, K. L., Roter, D. L., Biesecker, B. B., Cooper, L. A., & Erby, L. H. (2015). Genetic counselors' implicit racial attitudes and their relationship to communication. *Health Psychology*, *34*(2), 111–119. <https://doi.org/10.1037/hea0000155>

Scherr, C. L., Lindor, N. M., Malo, T. L., Couch, F. J., & Vadaparampil, S. T. (2015). Genetic counselors' practices and confidence regarding variant of uncertain significance results and reclassification from *brca* testing. *Clinical Genetics*, *88*(6), 523–529.

<https://doi.org/10.1111/cge.12563>

Slavin, T. P., Manjarrez, S., Pritchard, C. C., Gray, S., & Weitzel, J. N. (2019). The effects of genomic germline variant reclassification on clinical cancer care. *Oncotarget*, *10*(4), 417–423. <https://doi.org/10.18632/oncotarget.26501>

- Slavin, T. P., Van Tongeren, L. R., Behrendt, C. E., Solomon, I., Rybak, C., Nehoray, B., Kuzmich, L., Niell-Swiler, M., Blazer, K. R., Tao, S., Yang, K., Culver, J. O., Sand, S., Castillo, D., Herzog, J., Gray, S. W., & Weitzel, J. N. (2018). Prospective study of cancer genetic variants: Variation in rate of reclassification by ancestry. *JNCI: Journal of the National Cancer Institute*, *110*(10), 1059–1066. <https://doi.org/10.1093/jnci/djy027>
- Solomon, I., Harrington, E., Hooker, G., Erby, L., Axilbund, J., Hampel, H., Semotiuk, K., Blanco, A., Klein, W. M., Giardiello, F., & Leonard, L. (2017). Lynch syndrome limbo: Patient understanding of variants of uncertain significance. *Journal of Genetic Counseling*, *26*(4), 866–877. <https://doi.org/10.1007/s10897-017-0066-y>
- Starita, L. M., Ahituv, N., Dunham, M. J., Kitzman, J. O., Roth, F. P., Seelig, G., Shendure, J., & Fowler, D. M. (2017). Variant interpretation: Functional assays to the rescue. *The American Journal of Human Genetics*, *101*(3), 315–325. <https://doi.org/10.1016/j.ajhg.2017.07.014>
- Starita, L. M., Young, D. L., Islam, M., Kitzman, J. O., Gullingsrud, J., Hause, R. J., Fowler, D. M., Parvin, J. D., Shendure, J., & Fields, S. (2015). Massively parallel functional analysis of BRCA1 RING domain variants. *Genetics*, *200*(2), 413–422. <https://doi.org/10.1534/genetics.115.175802>
- U.S. National Library of Medicine. (2021, August 11). *Genetic/familial high-risk assessment: Breast, ovarian, and pancreatic, version 2.2021, NCCN clinical practice guidelines in oncology*. Journal of the National Comprehensive Cancer Network : JNCCN. Retrieved April 18, 2022, from <https://pubmed.ncbi.nlm.nih.gov/33406487/>

- Vears, D. F., Sénécal, K., & Borry, P. (2019). Genetic health professionals' experiences returning results from diagnostic genomic sequencing to patients. *Journal of Genetic Counseling*, 29(5), 807–815. <https://doi.org/10.1002/jgc4.1209>
- Vyas, D. A., Eisenstein, L. G., & Jones, D. S. (2020). Hidden in plain sight — reconsidering the use of race correction in clinical algorithms. *New England Journal of Medicine*, 383(9), 874–882. <https://doi.org/10.1056/nejmms2004740>
- Yadav, S., LaDuca, H., Polley, E. C., Hu, C., Niguidula, N., Shimelis, H., Lilyquist, J., Na, J., Lee, K. Y., Gutierrez, S., Yussuf, A., Hart, S. N., Davis, B. T., Chao, E. C., Pesaran, T., Goldgar, D. E., Dolinsky, J. S., & Couch, F. J. (2020). Racial and ethnic differences in multigene hereditary cancer panel test results for women with breast cancer. *JNCI: Journal of the National Cancer Institute*, 113(10), 1429–1433. <https://doi.org/10.1093/jnci/djaa167>

Supplement

Table 1.

Races and Ethnicities Included in Other

Race and Ethnicity
Cape Verdian
1/2 AJ
Aboriginal
African: Sudan
American
Assyrian
Australian
Belize
Belizean
Belizian
Brasilian

Brazil
Brazilian
Brazillian
Cape Verde
Cape Verde Creole
Cape Verde Islands
Cape Verdean
Cape Verdean (mixed African/Euro)
Cape Verdeans
Cape Veridian
Caucasian/ Jew
Caucasian/1/2 sephartic (Iraq)
Creole
Dominica
Dominican
Dominican Republic
Dominican Republic/Islander
Dominican Republican
Dr
Guyana/China
Guyanaese
Guyanan
Guyauese
Indigenous (Inuit)
Inuit
Iranian Jew
Iraqi/Iranian/Jewixh
Latin American (Brazilian)
Malagasay
Malagasy
Maori
Moorish
Moraccan
Morican
Morocan & Sudanese
Moroccan
Moroccan/North African
Morracan
Morrocan
Morroccan

Native
New Zealander
North African
North American
Northeast African
Other
Sefardic
Sepharaic
South Africa
South African
South America (Guyana)
South American/Guyana
Spanish Jew
St. Lucian/ Caribbean
Sudan
Sudanese
Sudanese Arab
Sudanese/Moroccan
Tajikistan
Trinidad
Trinidad
Trinidadian
Trinidadian/ Tobagonian
Trinidadians
Tunisia
Tunisian
West Indian
West Indies
White Persian
Indigenous/Belizean
Cape Verden
Native American and Other (Unknown)
English Jew, Caucasian
Other, not listed
Polish, non-AJ
Native American, Caucasian, European Jew

Table 2.*Missense and RING/BRCT variants in Individuals by Race and Ethnicity (% Total Tested)*

	Total	Black	Asian	Hispanic	Middle Eastern	NHW
Total Patients Tested	358721 ^a	27526	15568	25550	2154	217050
Reportable BRCA1 Variant	8036 (2.24%)	943 (3.43%)	650 (4.18%)	837 (3.28%)	83 (3.85%)	4635 (2.14%)
Missense Variant	3074 (0.86%)	490 (1.78%)	342 (2.20%)	330 (1.29%)	32 (1.49%)	1778 (0.82%)
RING/BRCT Variant	562 (0.16%)	90 (0.33%)	43 (0.28%)	75 (0.29%)	8 (0.37%)	346 (0.16%)

^aThis number represents all individuals tested during our study period, including those that did not self-report race/ethnicity as Black, Ashkenazi Jewish, Asian, Hispanic, Middle Eastern, and NHW.