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Diagnostic Outcomes of Concurrent DNA and RNA Sequencing in Individuals Undergoing Hereditary Cancer Testing

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Supplemental content

IMPORTANCE Personalized surveillance, prophylaxis, and cancer treatment options for individuals with hereditary cancer predisposition are informed by results of germline genetic testing. Improvements to genomic technology, such as the availability of RNA sequencing, may increase identification of individuals eligible for personalized interventions by improving the accuracy and yield of germline testing.

OBJECTIVE To assess the cumulative association of paired DNA and RNA testing with detection of disease-causing germline genetic variants and resolution of variants of uncertain significance (VUS).

DESIGN, SETTING, AND PARTICIPANTS Paired DNA and RNA sequencing was performed on individuals undergoing germline testing for hereditary cancer indication at a single diagnostic laboratory from March 2019 through April 2020. Demographic characteristics, clinical data, and test results were curated as samples were received, and changes to variant classification were assessed over time. Data analysis was performed from May 2020 to June 2023.

MAIN OUTCOMES AND MEASURES Main outcomes were increase in diagnostic yield, decrease in VUS rate, the overall results by variant type, the association of RNA evidence with variant classification, and the corresponding predicted effect on cancer risk management.

RESULTS A total of 43 524 individuals were included (median [range] age at testing, 54 [2-101] years; 37 373 female individuals [85.7%], 6224 male individuals [14.3%], and 2 individuals of unknown sex [<0.1%]), with 43 599 tests. A total of 2197 (5.0%) were Ashkenazi Jewish, 1539 (3.5%) were Asian, 3077 (7.1%) were Black, 2437 (5.6%) were Hispanic, 27 793 (63.7%) were White, and 2049 (4.7%) were other race, and for 4507 individuals (10.3%), race and ethnicity were unknown. Variant classification was impacted in 549 individuals (1.3%). Medically significant upgrades were made in 97 individuals, including 70 individuals who had a variant reclassified from VUS to pathogenic/likely pathogenic (P/LP) and 27 individuals who had a novel deep intronic P/LP variant that would not have been detected using DNA sequencing alone. A total of 93 of 545 P/LP splicing variants (17.1%) were dependent on RNA evidence for classification, and 312 of 439 existing splicing VUS (71.1%) were resolved by RNA evidence. Notably, the increase in positive rate (3.1%) and decrease in VUS rate (−3.9%) was higher in Asian, Black, and Hispanic individuals combined compared to White individuals (1.6%; *P* = .02; and −2.5%; *P* < .001).

CONCLUSIONS AND RELEVANCE Findings of this diagnostic study demonstrate that the ability to perform RNA sequencing concurrently with DNA sequencing represents an important advancement in germline genetic testing by improving detection of novel variants and classification of existing variants. This expands the identification of individuals with hereditary cancer predisposition and increases opportunities for personalization of therapeutics and surveillance.

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Applications of precision medicine are widespread, affecting clinical research, health care delivery systems, public health, and numerous specialty areas of patient care. Precision medicine has been adopted nowhere more than in oncology, where both tumor biomarkers and germline variants can be used to guide therapeutics and long-term management.[1,2] Germline genetic testing of cancer predisposition genes also identifies individuals at increased risk of developing cancer, which allows for risk stratification and personalized medical management.[3,4] Recommendations for cancer risk surveillance, prophylactic surgery, and in some cases radiotherapy can be influenced by genetic testing. As evidence supporting the efficacy of specific systemic therapy in individuals with germline pathogenic variants (PVs) grows, so does the importance of comprehensive identification of eligible patients.[5-7] Familial cascade testing can further expand the benefit of genetic testing by identifying other at-risk individuals eligible for early interventions.[8]

Advances in genetic testing technology, such as development of next-generation sequencing and methodologies to identify gross deletions and duplications, have improved the accuracy of germline genetic testing and the ability to identify individuals with hereditary cancer risk.[9,10] In recent years, increased adoption of multigene panel tests (MGPTs), rather than a targeted, gene-specific, stepwise approach to testing, has further expanded the DNA-based identification of germline cancer susceptibility.[11-13] While initial efforts to expand precision medicine have focused on DNA-based technologies, its full potential cannot be realized without the context of the RNA transcriptome.[14,15] DNA is transcribed into RNA, and before being translated into protein, it is spliced in messenger RNA (mRNA), and the pathogenicity of a variant can stem from impacts to mRNA splicing. Incorporating analysis of mRNA further bolsters accuracy of genetic testing by providing a functional context for variants identified at the DNA level that may impact RNA splicing.[16,17] In fact, it has been previously shown that the splicing profile generated by RNA sequencing of 18 cancer predisposition genes can detect missplicing by comparing mRNA transcript profiles from control data sets to transcripts from whole blood of individuals with pathogenic germline splicing variants in these genes.[17] This indicates that paired germline DNA and RNA sequencing holds great potential and provides a new opportunity for the identification of individuals with germline cancer predisposition.

Building off prior work in which RNA sequencing was performed reflexively on a small cohort of select individuals and variants at our laboratory,[18] here we assessed the diagnostic outcomes in 43 524 consecutive individuals undergoing concurrent DNA and RNA sequencing for hereditary cancer predisposition to measure the impact on positive yield and variants of uncertain significance (VUS) rate. We describe the association of splicing variants with hereditary cancer susceptibility and assess the association of RNA sequencing over time with the identification of PVs. We also evaluated the subsequent resolution of VUS in a cohort of 500 000 additional individuals tested at our laboratory and discussed the associated clinical implications.

Key Points

Question In what ways can adding RNA sequencing to germline genetic testing improve accuracy and clinical sensitivity over time compared to DNA sequencing alone?

Findings In this diagnostic study including 43 524 individuals undergoing hereditary cancer testing, RNA sequencing was simultaneously associated with increased diagnostic positive yield and decreased inconclusive rate of multigene panel testing for germline cancer predisposition. RNA-dependent classifications were made in 17.1% of splicing variants classified as pathogenic (1.9% of any variant classified as pathogenic), and 71.1% of splicing variants of uncertain significance (2.1% of any variants of uncertain significance) resolved as benign.
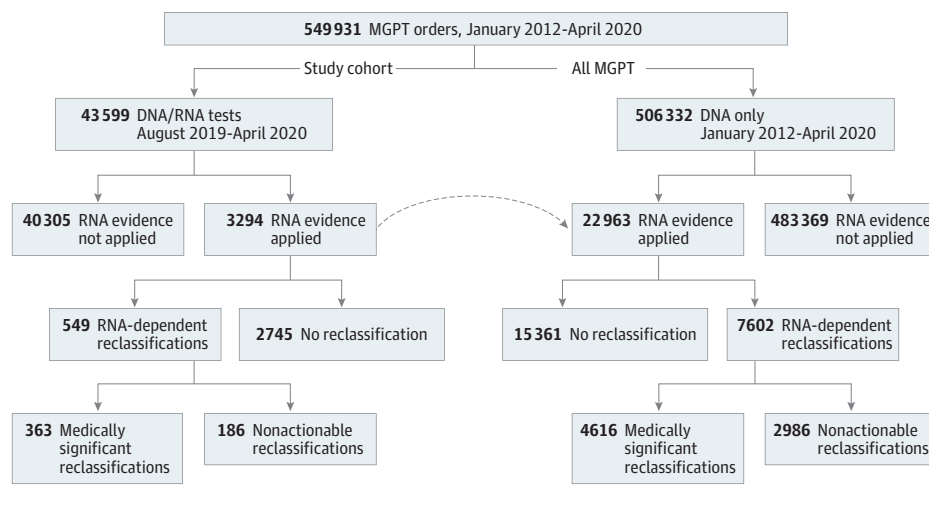
Meaning RNA sequencing provides an opportunity to more accurately identify individuals with hereditary cancer susceptibility by increasing detection and improving classification of disease-causing variants.

Methods

Study Population

Clinical data and molecular results from individuals who underwent paired DNA and RNA genetic testing for hereditary cancer predisposition at a clinical diagnostic laboratory (Ambry Genetics) from March 2019 through April 2020 were reviewed. While RNA sequencing was performed in all cases, the majority had mRNA transcript profiles consistent with controls and were not predicted to have a potential splicing variant. After RNA analyst review, these cases were reported with respect to the DNA results and interpretation (workflow in eFigure in Supplement 1). Individuals with MGPT including at least 1 of 18 genes covered by RNA sequencing were analyzed (*APC*, *ATM*, *BRCA1*, *BRCA2*, *BRIP1*, *CDH1*, *CHEK2*, *MLH1*, *MSH2*, *MSH6*, *MUTYH*, *NF1*, *PALB2*, *PMS2*, *PTEN*, *RAD51C*, *RAD51D*, and *TP53*). eTable 1 in Supplement 1 supplies a comprehensive list of tests and genes included in this study. All data presented were obtained as part of standard diagnostic testing. WGC IRB (formerly Western Institutional Review Board) determined the study to be exempt from the Office for Human Research Protections Regulations for the Protection of Human Subjects (45 CFR 46). Demographic and clinical information including sex assigned at birth, self-reported race and ethnicity, age, tumor type, and age at diagnosis were collected from the test requisition form and supporting clinical documents provided by the ordering clinician. Race, ethnicity, and ancestry were self-reported by patients and obtained from test-requisition forms including the following options: Ashkenazi Jewish, Asian, Black, Hispanic, White, other, and unknown. Specific information for other was not captured and not included, as it contains hundreds of specific denominations. Demographic information such as race and ethnicity was collected to aid in variant assessment. Deidentified data were curated as samples were received. We evaluated the overall results by variant type, the effect of RNA sequencing on variant classification, and the potential impact on medical manage-

Figure 1. Study Flowchart

549 931 MGPT orders, January 2012-April 2020

Study cohort — All MGPT

43 599 DNA/RNA tests
August 2019-April 2020

506 332 DNA only
January 2012-April 2020

40 305 RNA evidence
not applied

3294 RNA evidence
applied

22 963 RNA evidence
applied

483 369 RNA evidence
not applied

549 RNA-dependent
reclassifications

2745 No reclassification

15 361 No reclassification

7602 RNA-dependent
reclassifications

363 Medically
significant
reclassifications

186 Nonactionable
reclassifications

4616 Medically
significant
reclassifications

2986 Nonactionable
reclassifications

Individuals included in the concurrent DNA/RNA sequencing study are reflected in the left side of the diagram. All patients tested via multigene panel testing (MGPT) from January 2012 through April 2020 with DNA-only sequencing are reflected in the right side of the diagram.

ment. Data reporting was performed using the Standards for Reporting of Diagnostic Accuracy (STARD) reporting guideline.

DNA and RNA Sequencing and Interpretation

DNA sequencing, deletion and duplication analysis, and RNA analysis were performed as described previously.[19] Classification of sequence variations was based on the American College of Medical Genetics and Genomics and the Association for Molecular Pathology (ACMG/AMP) guidelines.[20] Variants were classified as pathogenic (P), likely pathogenic (LP), both considered positives or clinically actionable classifications, and VUS, likely benign (LB), or benign (B) according to a 5-tier variant classification protocol.[21] A recent publication from the ClinGen Sequence Variant Interpretation splicing subgroup provides clarifications and recommendations regarding the use of ACMG/AMP evidence codes relating to variant location, splicing predictions, splicing assay data, and variant type to capture splicing-related evidence and help standardize variant pathogenicity classification processes when interpreting RNA-based evidence.[22] Our approach to using RNA data to inform variant classification was aligned with these recommendations and involved considerations of the ultimate impact of the splice disruption on the protein as well as the magnitude, specificity, and reproducibility of the splicing disruption. SpliceAI in silico modeling was also used to predict splicing impact of variants.[23] We defined potential splicing variants as variants with a SpliceAI score of 0.5 or greater, any intronic variants within 5 nucleotides of an exon regardless of SpliceAI score, and any variant associated with an abnormal RNA transcript, regardless of the nucleotide position. We defined an abnormal RNA transcript as any RNA transcript whose sequence composition or abundance was different than that seen in a control data set, as previously described.[19] In summary, the relative expression of splicing events is measured by percent splicing index. The number or type of splicing events detected in patients was compared to a control data set to identify abnormal transcripts expressed above the control's threshold.

Impact of RNA on Variant Classification

Changes in classification of variants were prospectively tracked over time. Variant classifications at the onset of the study were recorded, and variants with RNA evidence were collated at the close of study. We refer to cases as RNA evidence applied (Figure 1) as those with either (1) an abnormal RNA transcript that met quality metrics to be leveraged toward variant interpretation or (2) a lack of abnormal RNA transcript associated with a potential spliceogenic variant that was informative for variant interpretation. Furthermore, variants with RNA evidence applied include those in which the evidence did not lead to a reclassification (ie, RNA evidence was concordant with an existing classification of pathogenic, or RNA evidence was insufficient to prompt a reclassification). Conversely, we defined RNA-impacted variants only as those with a change in classification due to RNA evidence. This included reclassifications that did not change the clinical actionability of a variant. Therefore, variants that were reclassified from LP to P and LB to B were included. We also sought to determine the number of RNA-dependent reclassifications that affected eligibility for surveillance and surgical recommendations described by the National Comprehensive Cancer Network (NCCN) guidelines for genetic/familial high-risk assessment for breast, ovarian, and pancreatic cancer[24] and colorectal cancer.[25] These were defined as potentially clinically actionable reclassifications. Finally, we defined medically significant reclassifications as both potentially clinically actionable reclassifications and those in which VUS were downgraded to LB/B. Changes in classification were compared across racial and ethnic groups. All statistical analyses were performed using R, version 4.1.1 (R Foundation). Statistical tests, χ^2 or Fisher exact test, were 2-sided with $P \le .05$ considered statically significant.

Results

A total of 43 599 tests from 43 524 consecutive individuals (median [range] age at testing, 54 [2-101] years) who under-

Table 1. Demographic Characteristics of Study Group

Characteristic	No. (%) (N = 43 599)
Sex assigned at birth	
Female	37 373 (85.7)
Male	6224 (14.3)
Unknown	2 (<0.1)
Age at testing, median (range), y	54 (2-101)
Race, ethnicity, and ancestry[a]	
Ashkenazi Jewish	2197 (5.0)
Asian	1539 (3.5)
Black	3077 (7.1)
Hispanic	2437 (5.6)
White	27 793 (63.7)
Other	2049 (4.7)
Unknown	4507 (10.3)

[a] Race, ethnicity, and ancestry were self-reported by patients and obtained from test-requisition forms including the following options: Ashkenazi Jewish, Asian, Black, Hispanic, White, other, and unknown. Specific information for other was not captured and not included, as it contains hundreds of specific denominations.

Table 2. Cancer History of Study Group

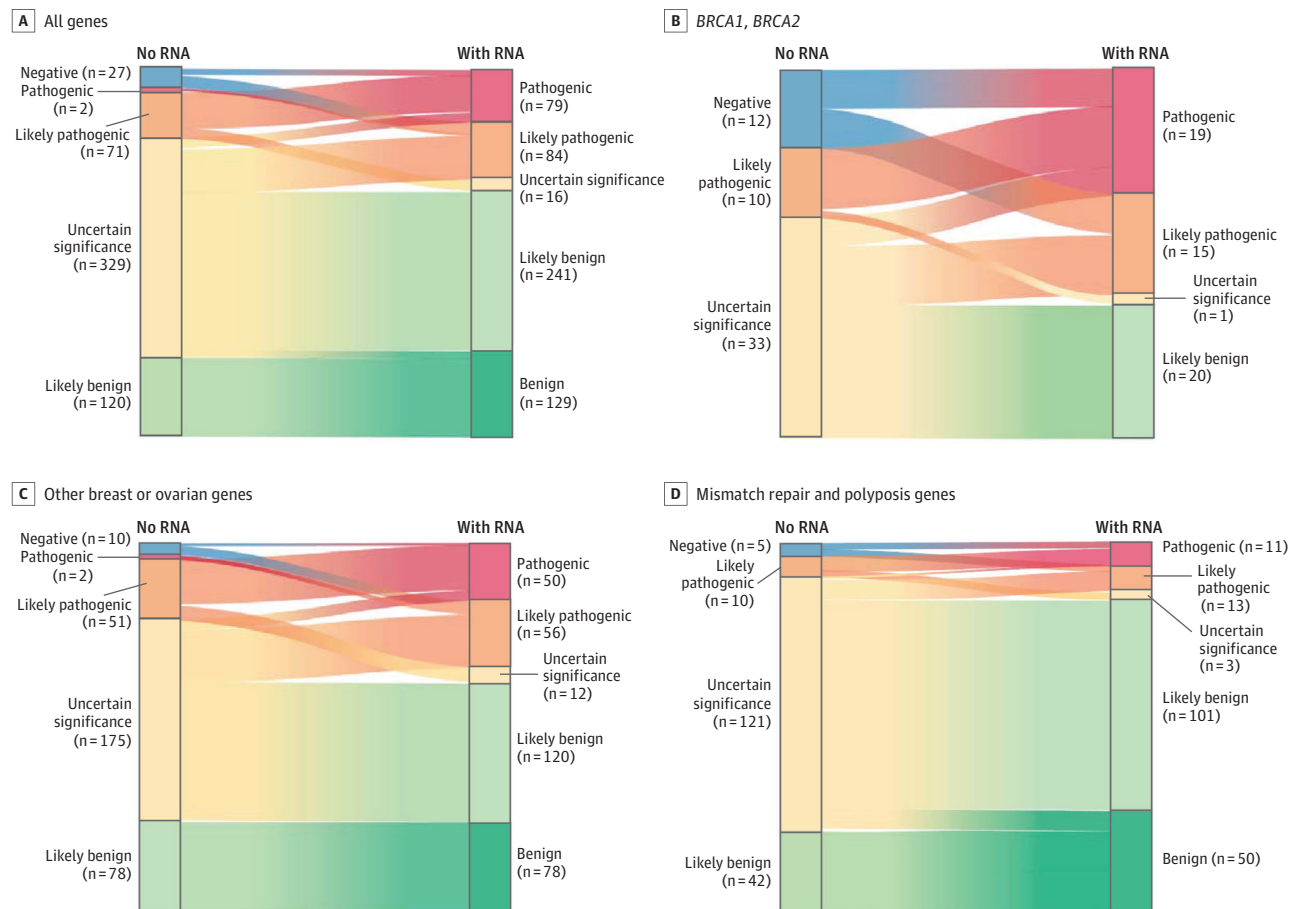| Cancer history	No. (%)[a] (N = 43 599)
Personal history of cancer	
Breast	17 021 (39.0)
Colorectal	2113 (4.9)
Ovarian	1926 (4.4)
Prostate	1659 (3.8)
Pancreatic	1433 (3.3)
Melanoma	1283 (2.9)
Uterine/endometrial	1211 (2.8)
Kidney	672 (1.5)
Thyroid	660 (1.5)
Other	3779 (8.7)
Met NCCN personal history criteria	10 837 (24.9)
No personal history of cancer	15 195 (34.9)

Abbreviation: NCCN, National Comprehensive Cancer Network.

[a] Does not equal 100%, as some individuals had more than 1 cancer.

went paired DNA-RNA genetic testing were eligible for this study. Most patients had test orders that included all 18 genes with available RNA coverage (35 145 of 43 599 [80.6%]; mean [SD] number of RNA-covered genes included, 17 [2.5]). The cohort was predominantly female (37 373 [85.7%]) (**Table 1**). Of the 43 599 patients, 2197 (5.0%) were Ashkenazi Jewish, 1539 (3.5%) were Asian, 3077 (7.1%) were Black, 2437 (5.6%) were Hispanic, 27 793 (63.7%) were non-Hispanic White (hereafter referred to as White), and 2049 (4.7%) were other race, and for 4507 individuals (10.3%), race and ethnicity were unknown. A total of 28 404 individuals (65.1%) had a personal history of cancer (**Table 2**). Breast cancer was the most frequent cancer type, reported in 17 021 individuals (39.0%), followed by colorectal (2113 [4.9%]) and ovarian (1926 [4.4%]). Overall, 10 837 individuals (24.9%) met NCCN criteria for testing based on personal history of cancer (Table 2). Positive results (P/LP variants) were reported in 11.2% of individuals (5130 PVs in 4873 individuals), and VUS were reported in 20.1% (9912 VUS in 8762 individuals). Among those who had paired DNA-RNA testing, RNA evidence was applied to 586 unique variants in 3294 individuals (Figure 1). In most paired DNA-RNA cases, the RNA evidence that was applied was concordant with an existing variant classification but did not prompt reclassification (2562 of 3294 [77.8%]) (ie, aberrant splicing was identified associated with a variant already classified as pathogenic). In some cases, RNA evidence was applied but was not sufficient to reclassify a VUS to LB/B or LP/P (183 of 3294 [5.6%]).

Evidence obtained from RNA sequencing impacted variant classification in 549 individuals who had paired RNA-DNA testing. The highest proportion of RNA-impacted cases was observed in individuals whose test order included only 1 RNA-covered gene (6 of 128 single RNA gene orders [4.7%]). Otherwise, the proportion or RNA-impacted cases remained fairly stable despite an increasing number of genes included in RNA sequencing (16 of 1746 orders with 2-10 RNA-covered genes [0.9%]; 542 of 41 715 orders with 11-18 RNA-covered genes [1.3%]). We evaluated the diagnostic outcomes before and af-

ter application of RNA evidence, depicted in **Figure 2**. RNA evidence strengthened a classification of LP and LB in 186 of the 549 impacted cases (33.9%) (LB to B, LP to P), leading to reclassification to P or B. Additionally, VUS were reclassified as LB or B in 250 impacted cases (45.5%). Medically significant reclassifications, which include upgrades and downgrades of alterations that would be classified as VUS without RNA, were made in 402 RNA-impacted cases (73.2%, 0.9% of the overall tested). Specifically, VUS downgrades were made in 305 cases, and potentially clinically actionable upgrades were made in 97 cases, including 70 individuals with VUS reclassified to P/LP and 27 individuals with deep intronic variants that were previously unreported and would not have been detected using DNA sequencing alone (Figure 2A). Notably, novel deep intronic P/LP variants made up 21.8% of the RNA-impacted variants in *BRCA1/2* (Figure 2B). An additional 41 individuals had intronic variants that had been previously classified as P/LP and were therefore not included as RNA-impacted variants.

In other breast/ovarian cancer genes (excluding *BRCA1/2*) where variant classification evidence derived from clinical history is often uninformative due to moderate penetrance nature of these genes, RNA sequencing supplied evidence that led to upgrades from VUS to P/LP in 17.5% of RNA-impacted cases (Figure 2C). Conversely, in the mismatch repair and polyposis genes, the majority of impacted variants were downgraded from VUS to B/LB (60.8% using RNA evidence) (Figure 2D). When RNA-dependent reclassifications across all genes were aligned with NCCN guidelines, we found that reclassifications were made in genes with recommendations for increased surveillance in 78 (14.2%) and surgical options in 32 RNA-impacted cases (5.8%). Medically significant downgrades from LP to VUS were made in 16 individuals, 13 of which were in genes with prophylactic surgical recommendations.

These RNA-impacted cases improved the positive rate and decreased the VUS rate of MGPT (**Figure 3**). Overall, the relative increase in diagnostic yield was 1.9%, indicating that 1 in 54 individuals with P/LP variants would have received results with incorrectly classified or undetected variants

Figure 2. Sankey Diagrams Depicting Change in Variant Classifications Based on RNA Evidence

A All genes

B *BRCA1, BRCA2*

C Other breast or ovarian genes

D Mismatch repair and polyposis genes

Comparisons of variant classifications at the onset of the study before RNA evidence was available (left side of diagrams) with variant classifications after RNA evidence was applied (right side of diagrams). A, Change in variant classifications based on RNA evidence in all genes together. B, Change in variant classifications based on RNA evidence in *BRCA1* and *BRCA2*. C, Change in variant classifications based on RNA evidence in other breast/ovarian genes: *BRIP1, CDH1, CHEK2, NF1, PTEN, PALB2, RAD51C, RAD51D,* and *TP53*. D, Change in variant classifications based on RNA evidence in mismatch repair and polyposis genes: *APC, MLH1, MSH2, MSH6, MUTYH,* and *PMS2*.
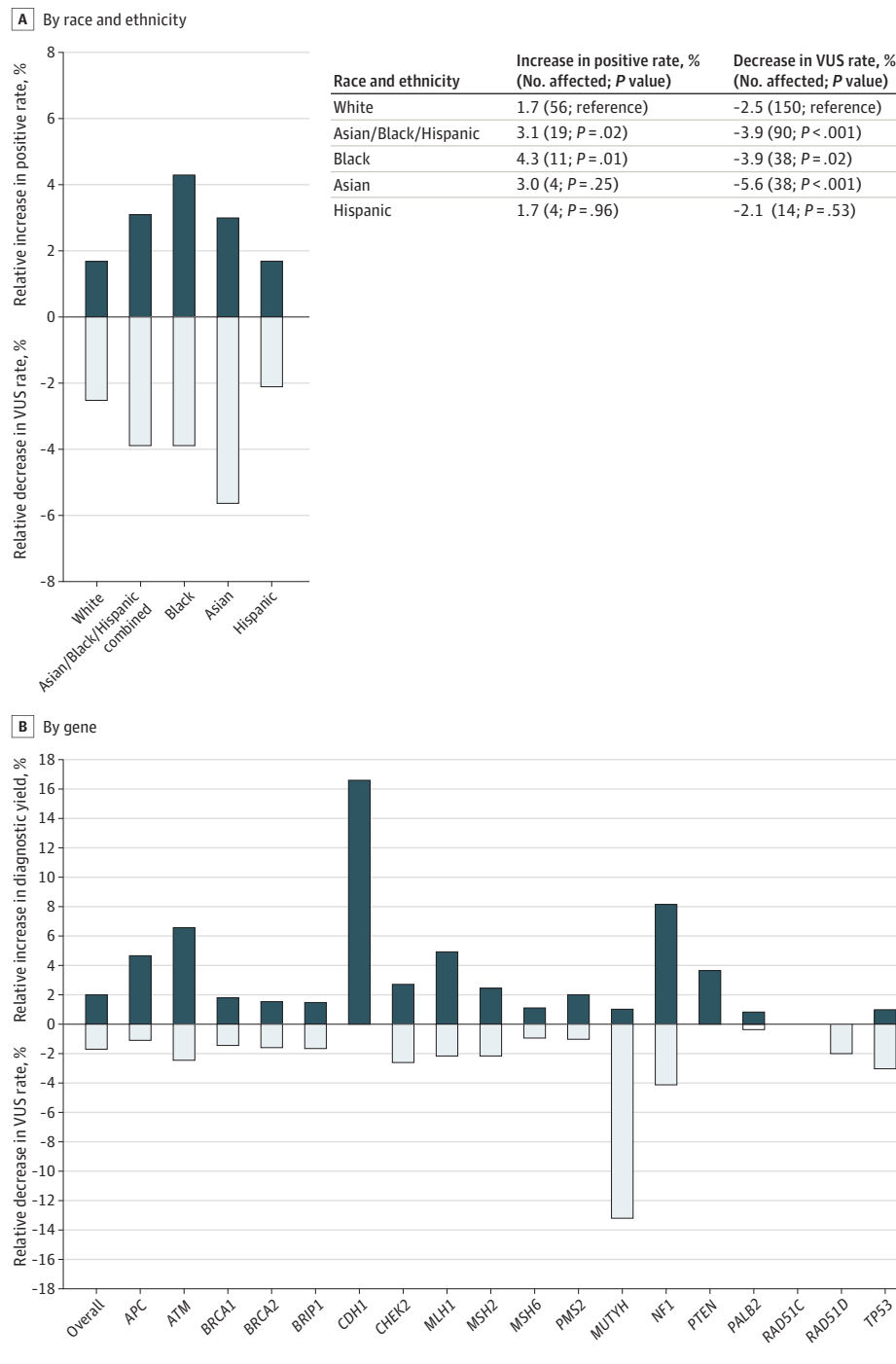
without the addition of RNA evidence. The impact on positive and VUS rate varied by racial and ethnic group (Figure 3A). In Asian, Black, and Hispanic individuals combined, positive rate increased by 3.1%, and VUS rate decreased by 3.9%. These changes were greater than the increase in PV rate (1.6%; P = .02) and decrease in VUS rate (−2.5%; P < .001) observed in White individuals. These trends were observed when comparing individual racial and ethnic groups, though they were not always statistically significant due to smaller sample size.

We also evaluated differences in positive and VUS rate by gene (Figure 3B). The relative increase in positive rate was more than 5% in *ATM, CDH1, MLH1,* and *NF1*. Along with the increase in yield, the VUS rate decreased in nearly all genes, most notably in *CHEK2, MUTYH, NF1,* and *TP53*. The relative decrease in VUS rate overall was 1.8%. As expected, RNA sequencing was especially impactful on yield and VUS rate for potential splicing variants, where we observed that 93 of 545 P/LP splicing variants (17.1%) were dependent on RNA evi-

dence, and 312 of 439 existing splicing VUS (71.1%) were resolved and classified as LB/B.

We observed that the majority of RNA-impacted cases (75.7% of P/LP and 73.6% of B/LB) had recurrent variants observed in more than 1 individual, indicating that many RNA-impacted variants are recurrent in the population. This was further demonstrated when we evaluated the subsequent resolution of VUS in a cohort of 506 332 additional individuals tested at our laboratory through April 2020 (Figure 1). This includes individuals tested before and during the study period who did not order RNA sequencing. Using current guidelines,[20,22] once a variant is reclassified, the classification can be applied to all individuals with that variant. A total of 22 963 individuals with DNA-only testing (4.5%) were found to carry a variant with relevant RNA evidence generated from the 43 524 individuals included in this study. Reclassifications were made in 7602 of these 22 963 individuals, and updated reports were sent to ordering clinicians.

Figure 3. Improvement in Positive and Variant of Uncertain Significance (VUS) Rates

A By race and ethnicity

Race and ethnicity	Increase in positive rate, % (No. affected; P value)	Decrease in VUS rate, % (No. affected; P value)
White	1.7 (56; reference)	-2.5 (150; reference)
Asian/Black/Hispanic	3.1 (19; P = .02)	-3.9 (90; P < .001)
Black	4.3 (11; P = .01)	-3.9 (38; P = .02)
Asian	3.0 (4; P = .25)	-5.6 (38; P < .001)
Hispanic	1.7 (4; P = .96)	-2.1 (14; P = .53)

B By gene

A, Relative increase in positive rate and decrease in VUS rate by race and ethnicity. B, Relative increase in positive rate and decrease in VUS rate by gene.

Discussion

Germline genetic testing has inherent uncertainty. While VUS are often discussed, another major source of uncertainty is the accuracy of variant classifications. Our results demonstrate that paired DNA and RNA sequencing is associated with improved identification of individuals with a hereditary cancer predisposition. We observed that patho-genic splicing variants were not uncommon, indicating significant clinical utility for RNA sequencing. We found that RNA sequencing identified previously undetected PVs, resolved existing inconclusive results, and strengthened confidence in previous clinically actionable classifications made with more limited evidence, moving classifications from LP to P. Using classification rules based on published guidelines, upgrades from LP to P equate to an increase from 90% to greater than 99% probability of pathogenicity,[20,26,27]

which can increase confidence in management decision-making.

Clinical laboratories continue to make improvements to their assays to increase the clinical sensitivity of MGPT. The addition of newly characterized genes to expand DNA cancer panel size and the inclusion of testing methods to detect gross deletions/duplications increase the positive rate but also increase the VUS rate.[28] Limiting reported findings to known functional domains or specific types of variants can decrease the VUS rate but does not affect the positive rate. As previously suggested,[29] data presented here demonstrate that RNA sequencing is a unique assay improvement that is associated with both decreased VUS rate and increased positive rate. RNA sequencing may prove to be even more impactful than gross deletion/duplication testing, as splicing variants are more than twice as common as gross deletions and duplications.[19]

We found that RNA sequencing was particularly beneficial in underrepresented populations. Higher VUS rates in racial and ethnic minority populations have been widely reported.[30-32] Lack of representation of racial and ethnic minority individuals in testing cohorts, published literature, and population databases contributes to this disparity, as it limits the availability of evidence available in variant classification. However, performing concurrent DNA and RNA testing generates novel functional evidence to help fill existing gaps. As a result, RNA evidence had a greater impact on PV and VUS rate in racial and ethnic minority individuals in our cohort and therefore may play an important role in mitigating disparities in MGPT results reported in these groups.

Increased accuracy corresponds directly to medical management, as clinicians report changes to cancer risk recommendations and interventions in patients with positive results via MGPT.[33-36] Published management or consensus guidelines exist for all 18 genes covered by RNA sequencing in this study, so improvements in test accuracy correspond directly to clinical utility.[24,25] Moreover, our observation that recurrent variants observed in more than 1 individual made up three-quarters of RNA reclassifications indicates that reclassifications of variants via RNA sequencing can have a ripple effect with the potential for multiple individuals to receive an updated diagnostic outcome. Our prior work evaluating splicing outcomes in a hereditary cancer cohort was limited to individuals with paired DNA-RNA testing ordered and did not assess impact among the entire cohort undergoing germline genetic testing.[19] Here, we extend our analysis to calculate the impact on individuals during this study period who had DNA-only testing ordered, as well as those tested before paired DNA-RNA testing was initiated. Similarly to how evidence from externally published clinical or functional studies can be used to classify a variant in an unrelated patient, RNA data obtained from 1 individual can be applicable to other individuals with the same variant. Therefore, even when RNA evidence is used in a minority of cases, the benefit can translate to a large number of individuals in the setting of a high-volume diagnostic laboratory. In this way, RNA reclassifications cannot only resolve cases of missing heritability in familial cancer but also introduce opportunities for new therapeutic interventions and prophylaxis.

Limitations

This study has limitations. While we have shown that paired DNA and RNA sequencing is associated with improved detection rate and test accuracy, increasing access to novel technologies can be challenging, particularly due to added costs to laboratories. Future health economic studies will be important to help guide widespread implementation by health systems and ensure accessibility to all patients. In addition, this study was limited by the sample size and number of genes investigated. Despite its scale, there are analyses that would benefit from larger observations, such as gene-specific yield in conditions where PVs are exceedingly rare, or improved power in comparisons between racial and ethnic groups. Also, since genes with consensus or expert panel cancer risk management guidelines were prioritized for paired DNA and RNA sequencing, this study was limited to 18 genes. Formal study on additional predisposition genes will provide a more comprehensive understanding of the contribution abnormal splicing has on hereditary cancer syndromes.

Conclusions

This diagnostic study highlights the importance of RNA sequencing in precision medicine to improve the identification of high-risk individuals missed by DNA-only diagnostic approaches and the medical management of tested patients. The development of benchmarks for performance metrics and evidence weighting will expand the opportunity to apply RNA sequencing in clinical settings.[37] Data sharing and collaboration between researchers, laboratories, and clinicians will facilitate these efforts in the shared goal of improved patient outcomes.

Genetics) and were involved in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication, as described above in the Author Contributions.

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Data Sharing Statement: See Supplement 2.

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