Ambry Genetics®

WHITE PAPER

The Growing Impact of Concurrent DNA and RNA Sequencing

Introduction

Germline genetic testing of cancer predisposition genes may identify individuals at increased risk of developing cancer, which allows for risk stratification and personalized medical management.[1,2] 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.[3,4]

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. Germline pathogenic variants in non-coding regions (introns) of hereditary cancer genes are well known to cause cancer predisposition[5,6]; however, DNA sequencing of these large regions is cost-prohibitive and ultimately would result in identification of many inconclusive results that do not raise the positive yield. Ambry has developed and validated a novel, scalable assay—+RNAinsight™—which leverages paired DNA and RNA sequencing to identify clinically-actionable genetic variants in coding and non-coding regions[7,8] of 91 cancer predisposition genes, resulting in a significant increase in the positive yield of genetic testing.

Providers Recognize the Utility of +RNAinsight™

+RNAinsight™ is the first clinically available test to add concurrent RNA sequencing analysis to traditional DNA multigene panel testing for hereditary cancer.[9] RNA sequencing has been shown to increase diagnostic yield while simultaneously decreasing VUS rate. Providers have responded to this improved accuracy, as reflected in ordering trends since the inception of +RNAinsight™ in August 2020 (Figure 1).

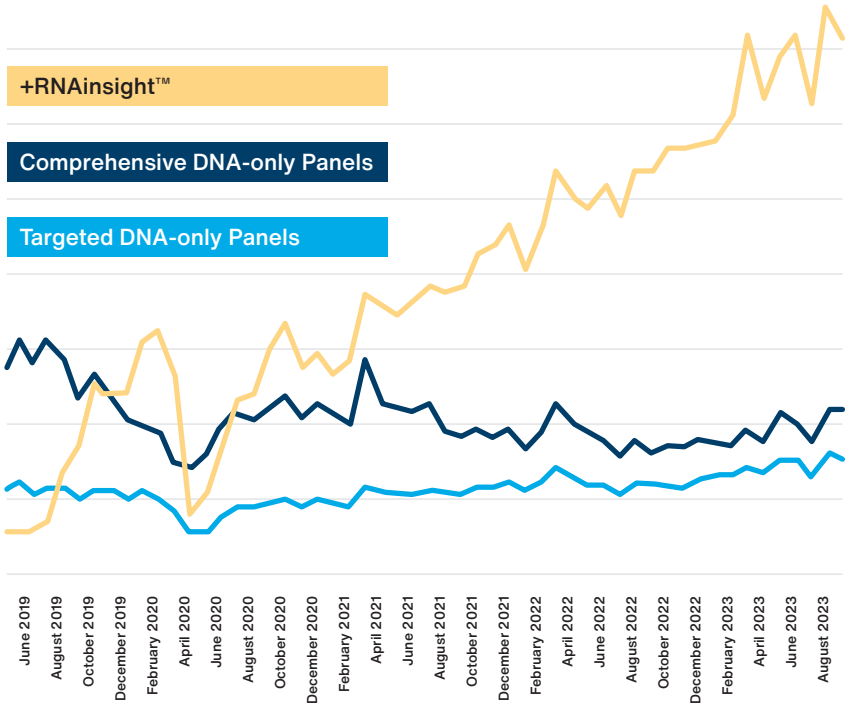

Figure 1. Ordering Trends Over Time

The Ripple Effect of +RNAinsight™

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 one 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. Therefore, **evidence generated from the first year of testing alone was applied to 26,000 individuals, or 5% of all individuals who had hereditary cancer panel testing at Ambry** (Figure 2).[10] This led to reclassifications that were dependent on RNA in 8,000 individuals.

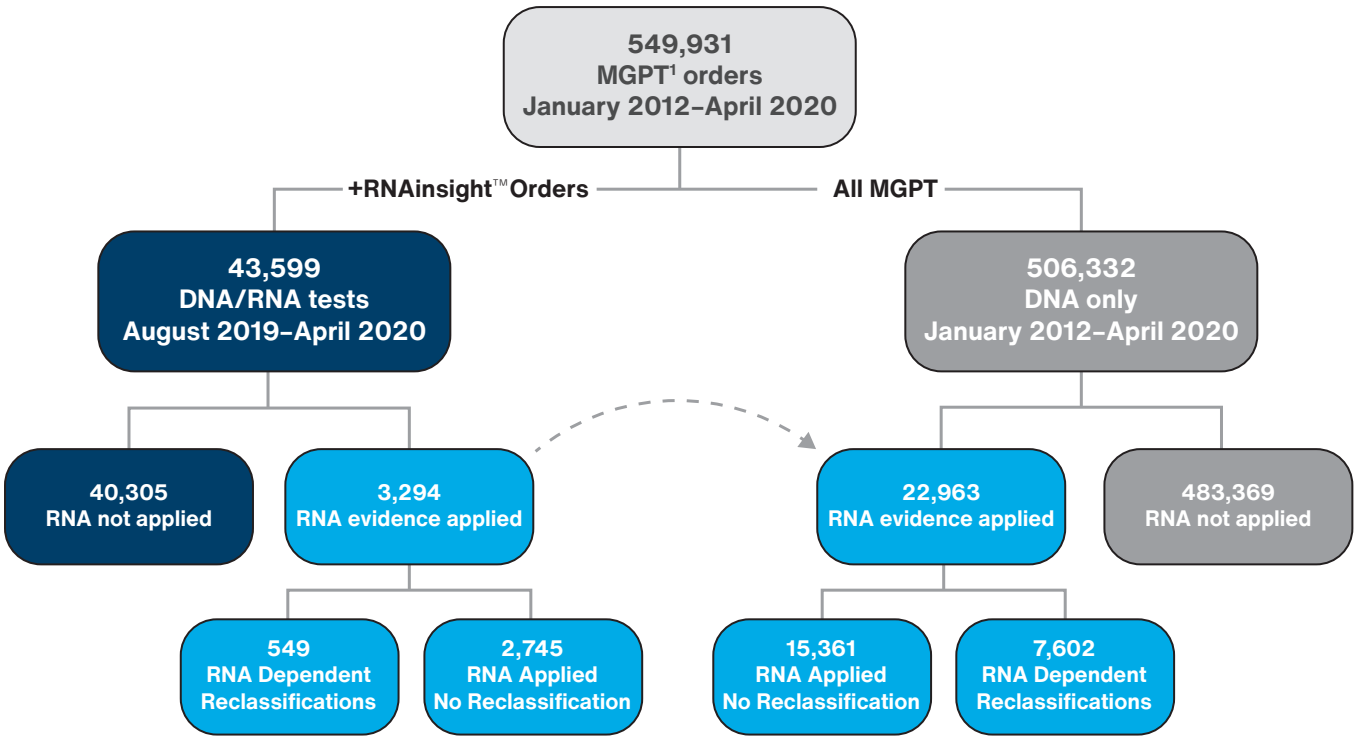

Figure 2. RNA Impact Flowchart
Individuals who had paired DNA-RNA testing at Ambry are depicted in the left side of the diagram. Individuals who had DNA-only testing at Ambry are depicted in the right side.

Applying RNA Evidence to a Variant Classification Framework

Assigning clinical significance to RNA evidence requires consideration of numerous variables (Figure 3). Ambry was invited to participate in the development of interpretation guidelines put forth by the ClinGen Sequence Variant Interpretation splicing subgroup regarding the use of ACMG/AMP evidence codes relating to variant location and type, splicing predictions, splicing assay data.[11] These guidelines will help standardize classification processes when interpreting RNA evidence across the industry.

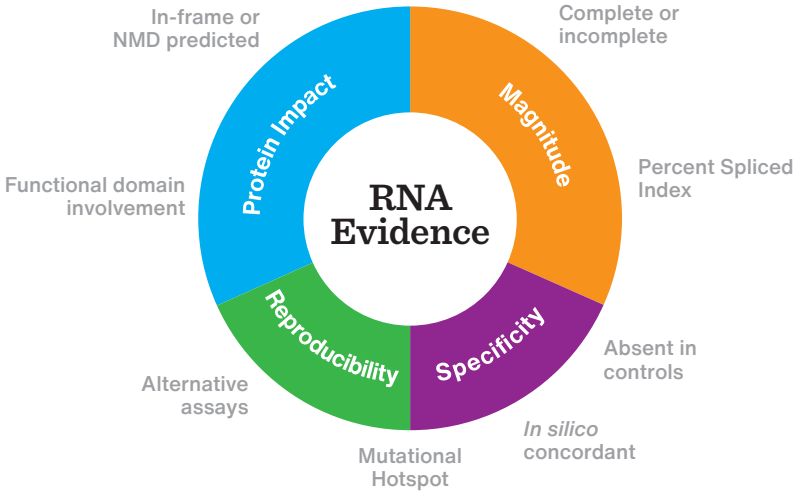

Figure 3. Evaluating quality of RNA evidence
Factors needed to be considered when determining how to apply RNA evidence for variant interpretation.

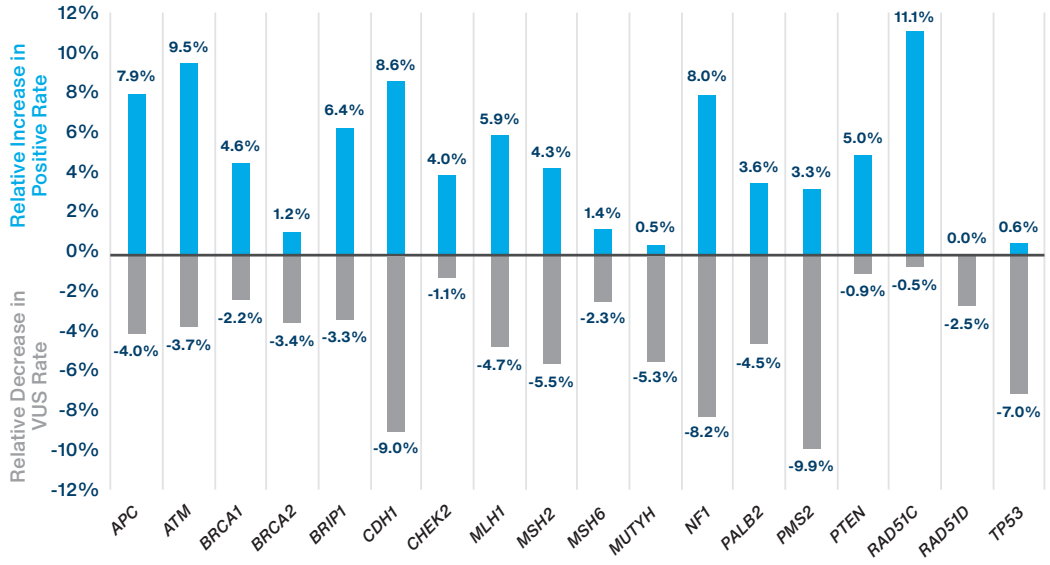

Figure 4. Impact on Positive and VUS Rate, by Gene.

Gene by Gene Impact

Several things can influence the utility of RNA sequencing in any given gene, including technical, clinical, and molecular factors. To this end, we have calculated the impact of RNA on diagnostic and inconclusive rate among 18 genes with clinical management guidelines (Figure 4). The relative increase in positive rate was approximately 5% or more in half these genes. Along with the increase in yield, the VUS rate decreased in all genes, most notably in *CDH1, NF1,* and *PMS2.* The relative decrease in VUS rate overall was 4.0%.

Mitigating Health Disparities

Genetic data used for variant classification are typically derived from European cohorts, so there are evidence gaps in underrepresented populations. This makes it more difficult to classify variants in these groups, which increases VUS rate and limits utility of testing. RNA sequencing generates novel functional evidence that helps close those gaps and leads to a preferential improvement in accuracy among non-White populations,

in which a larger increase and positive rate and decrease in VUS rate were recorded (Figure 5).

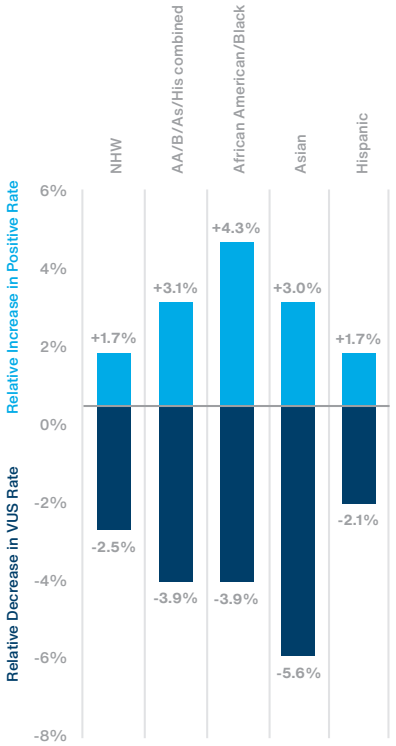

Figure 5. Impact on Positive and VUS Rate, by Race/Ethnicity

Race and ethnicity	Increase in positive rate, % (No. affected; *P* value)	Decrease in VUS rate, % (No. affected; *P* value)
White	1.7 (56; reference)	1.7 (56; reference)
Asian/Black/Hispanic	3.1 (19; *P*=.02)	3.1 (19; *P*=.02)
Black	4.3 (11; *P*=.01)	4.3 (11; *P*=.01)
Asian	3.0 (4; *P*=.25)	3.0 (4; *P*=.25)
Hispanic	1.7 (4; *P*=.96)	1.7 (4; *P*=.96)

Paired DNA and RNA sequencing may therefore play an important role in improving equity of genetic testing results. The increase in positive rate (3.1%) and decrease in VUS rate (−3.9%) was higher in Asian, Black, and Hispanic individuals combined undergoing DNA and RNA sequencing compared to White individuals (1.6%; P = .02; and −2.5%; P < .001).[10]

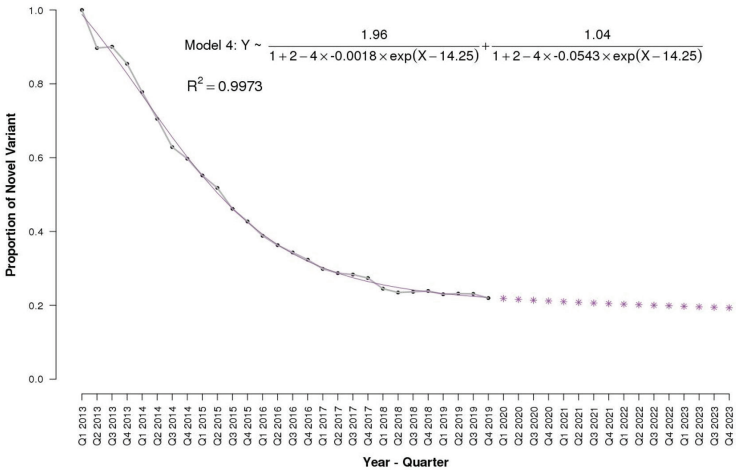

Figure 6. Proportion of Novel vs Recurrent Variants Over Time
Based on total number of actual variants observed each quarter, the rate of novel variants was found to stabilize at 20%.

Experience That Matters

In 2019, Ambry Genetics became the first clinical lab to introduce paired DNA-RNA testing. Since that time, we have performed concurrent DNA and RNA testing on over 800,000 patients and classified over 1,500 unique variants. We've grown our team to 34 scientists who analyze and interpret RNA results. To meet our continued commitment to data-sharing and transparency, we've contributed to over 30 scientific posters, presentations, and publications.

Long-Term Need for RNA Sequencing

When performing genetic testing in any given population, some variants will be observed over and over while others will be novel. Although existing RNA evidence can be used on recurrent variants, RNA sequencing still needs to be performed to detect and interpret novel variants. Due to the immense diversity in human genomic variation, we will essentially never exhaust the discovery of novel variants. Even after years of testing, novel variants make up about 20% of variants observed (Figure 6).

Conclusions

Concurrent DNA and RNA sequencing represents a paradigm shift in the standard of germline genetic testing. It:

- **increases the accuracy of variant interpretation**
- **improves detection of pathogenic variants**
- **resolves variants of uncertain significance**
- **addresses evidence gaps**
- **informs classification**

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