Ambry Genetics
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RESEARCH SUMMARY

Increasing Variant Resolution

AUGUST 2018

Recent studies highlight how additional testing can lead to increased resolution of variants of unknown significance (VUS), enabling us to provide clear answers to more patients.

DNA Breakpoint Assay (DBA) Informs Classification of Gross Duplications

KEY STUDY FINDINGS[1]

- Duplications of a large region of a gene (aka gross duplications) are often classified as variants of unknown significance (VUS).
- DBA was used to determine tandem* status of gross duplications in *ATM*, *BRCA1, BRCA2*, *CDH1*, *CHEK2*, and *PALB2* to inform variant classification.
- 21/22 (95%) unique duplications that were found to occur in tandem and were eligible for reclassifcation were upgraded to pathogenic or likely pathogenic providing clinically actionable results (Figure 2).
- DBA directly impacted 70 unique patients who now have clear genetic test results and can benefit from personalized medical management.

POINTS FOR YOUR PRACTICE

- Additional functional assays, such as DBA, can significantly improve variant resolution, leading to more clear results to better guide patient management.
- It is important to consider variant assessment expertise and capabilities when selecting a laboratory for genetic testing to decrease the chance of receiving an uncertain result and increase clarity for your patients.

** Duplications said to occur "in tandem" are located within the gene of question and may be more likely to cause a disruption*

Figure 1: Classifying Gross Duplications

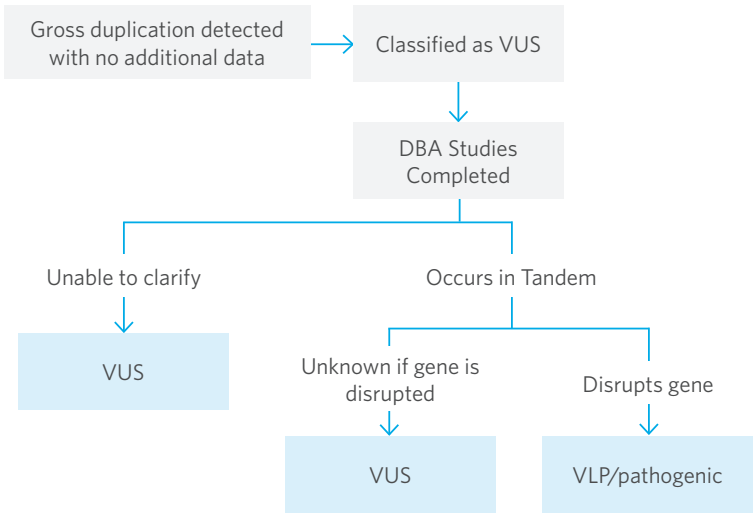
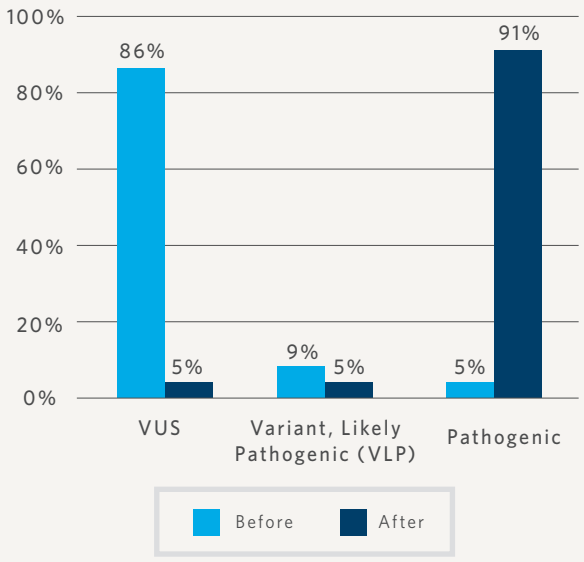


Figure 2: Reclassifaction of Eligible Tandem Duplations Before and After DBA



RESEARCH SUMMARY

Quantitative Analysis of *BRCA1* and *BRCA2* Germline Splicing Variants Using a Novel RNA-Massively Parallel Sequencing Assay

KEY FINDINGS[2]

- Genetic alterations with unclear effects on splicing are often categorized as VUS, which can be a challenge for healthcare providers and patients, as these are not clinically actionable results.
- Ambry developed CloneSeq, a novel, RNA-based massively parallel sequencing technique that enables us to determine a genetic variant's effect on splicing and better classify these potentially clinically actionable variants.
- To validate this assay we compared it to the RNA splicing assays recommended by members of the ENIGMA (Evidence-based Network for the Interpretation of Germline Mutant Alleles) consortium, and CloneSeq was able to replicate all findings.
- CloneSeq was also used to analyze blood samples obtained from carriers of *BRCA1* or *BRCA2* germline sequence variants, and enabled the classification of a novel *BRCA1* splicing variant.

COMPARISON OF AVAILABLE RNA SPLICING ASSAYS		
ENIGMA RNA Assays*	Real-time & Digital PCR	CloneSeq
Qualitative	Not qualitative	Qualitative ✓
Semi-quantitative	Quantitative	Quantitative ✓
Low-throughput		High-throughput ✓

POINTS FOR YOUR PRACTICE

- CloneSeq combined with Ambry's bioinformatics pipeline can allow for better classification of splicing variants, increasing the likelihood of clear, clinically actionable results.
- The ability to better classify splicing variants is fundamental for enabling personalized medical management recommendations for patients and their family members.

REFERENCES

1. Richardson *et al*. DNA Breakpoint Assay Reveals a Majority of Gross Duplications Occur in Tandem Reducing VUS Classifications in Breast Cancer Predisposition Genes. Genetics in Medicine. 27 July 2018.
2. Farber-Katz *et al*. Quantitative Analysis of BRCA1 and BRCA2 Germline Splicing Variants Using a Novel RNA-Massively Parallel Sequencing Assay. Front. Oncol., 27 July 2018.