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WHITE PAPER

Advancing Diagnostics through Genome Sequencing at Ambry Genetics

Introduction

Genomic testing is increasingly central to clinical medicine. Alongside the growing range of test indications, advances in technology and declining costs have expanded access to large-scale sequencing. Clinical practice has moved beyond targeted single-gene testing to broader gene panels and is progressively adopting comprehensive diagnostic genomic sequencing. Ambry's clinical diagnostic genome sequencing test, GenomeNext™, detects genomic variation across the genome to find answers for patients with rare disease.

Balancing Depth and Breadth: The Comparative Value of Exome and Genome Sequencing

Exome and genome sequencing are recommended first-tier diagnostic tests for patients with epilepsy, intellectual disability, developmental delay, and multiple congenital anomalies.[1-3] Exome and genome testing improve upon the breadth of genomic coverage and diagnostic yield of multi-gene panels and report fewer variants of uncertain significance (VUS).[4]

In contrast to gene panel tests where all variants in a pre-defined set of genes are reported, analysis for exome and genome sequencing is performed through the prioritized review and reporting of variants with phenotype overlap for a given patient. Additionally, the use of parental samples through trio-based testing provides the opportunity to further characterize variants of interest, reducing uncertainty, and improving diagnostic yield.

While both exome and genome sequencing are recommended, there are key differences. Exome sequencing captures 1-2% of the genome including all protein-coding regions, accounting for the majority of Mendelian disease described to date. Exome tests provide deep coverage across the exome to ensure confidence in identifying variants. In contrast, genome sequencing requires lower depth to provide more uniform coverage to reliably detect variants across the entire genome (coding and non-coding regions) (Figure 1). The expanded regions evaluated through genome sequencing support the identification of variant types that are not called from exome sequencing (Table 1).

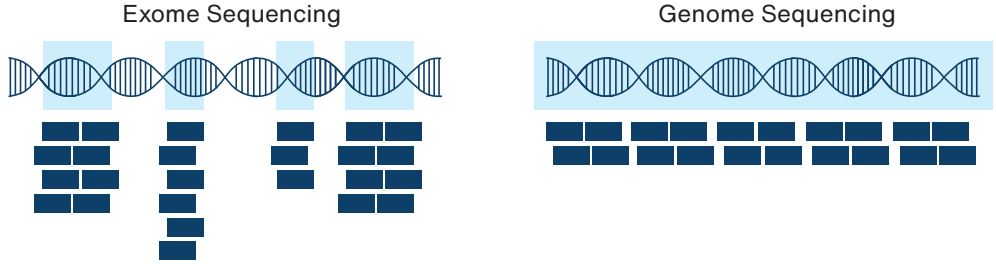

Figure 1. Depth and breadth of exome vs. genome sequencing.

Variant Type	Exome Sequencing	Genome Sequencing
SNVs in coding regions	✓	✓
Aneuploidy	✓	✓
Segmental mosaicism	✓	✓
CNVs	✓*	✓
STRs	✗	✓
Other SVs	✗	✓
Mitochondrial variants	✗	✓
Intronic and regulatory variants	✗	✓
Uniparental disomy	✗	✓
Non-protein coding genes	✗	✓

Table 1. Genome sequencing identifies more variant types than exome sequencing.

SNVs = single nucleotide variants; STRs = short tandem repeats; SVs = structural variants
* Exome sequencing can be limited in detection of copy number variants (CNVs). Additional or follow-up testing may be warranted in specific situations.

Importantly, genome sequencing identifies variants in non-coding genes, which have emerged as important to evaluate in patients with neurodevelopmental and epilepsy phenotypes. In the last few years, pathogenic variants in the snRNA genes, *RNU2-2* and *RNU4-2*, were identified to be among the most frequent causes of both dominant and recessive neurodevelopmental and epileptic disorders.[5-6]

RNU-opathies and genome sequencing

- Pathogenic variants in *RNU2-2* and *RNU4-2* represent an important cause of neurodevelopmental disorders related to abnormal pre-mRNA splicing.
- Biallelic pathogenic variants in *RNU2-2* cause the most prevalent known recessive neurodevelopmental disorder.
- Pathogenic variants in *RNU2-2* are associated with both dominant and recessive developmental and epileptic encephalopathies.
- Gene panels and exome sequencing tests generally do not detect *RNU2-2* and *RNU4-2* variants; genome sequencing is needed.

This expanded variant detection, in addition to the uniform coverage from genome sequencing data, increase diagnostic yield in patients who have genome testing vs. exome testing. Various studies have evaluated exome and genome sequencing diagnostic yield in patient cohorts with different phenotypes. The increase in diagnostic rate from genome sequencing varies depending on the date the exome sequencing was performed, the type of variants reported from exome sequencing, and the patient population being tested. In one study of patients with a high clinical suspicion of rare disease, the diagnostic rate from genome sequencing was 43% with 21% of these cases attributed to technically challenging variants identified via genome sequencing.[7] Another study in patients with rare disease phenotypes report up to a 43% diagnostic rate with 28% of these diagnoses identifiable via genome but not exome sequencing.[8]

Equity Through Innovation: The Power of the Pangenome

Ambry's genome advances equitable care by closing diagnostic gaps in historically underrepresented populations. GenomeNext leverages technology that integrates multigenome mapping and pangenome references. Using multiple mapping paths instead of a single linear reference improves test accuracy and ultimately provides more comprehensive genomic analysis (Figure 2).[9]

GRCh38 is the current linear human reference genome assembly that is widely used in genetic testing to align sequencing data and identify clinically relevant variants. Pangenome marks a transition from this linear approach to graphical reference sequencing. The Human Pangenome Research Consortium (HPRC), funded by the NIH, developed a human pangenome, a collection of many high-quality reference genomes that captures global genomic diversity. This work addresses limitations in the current reference

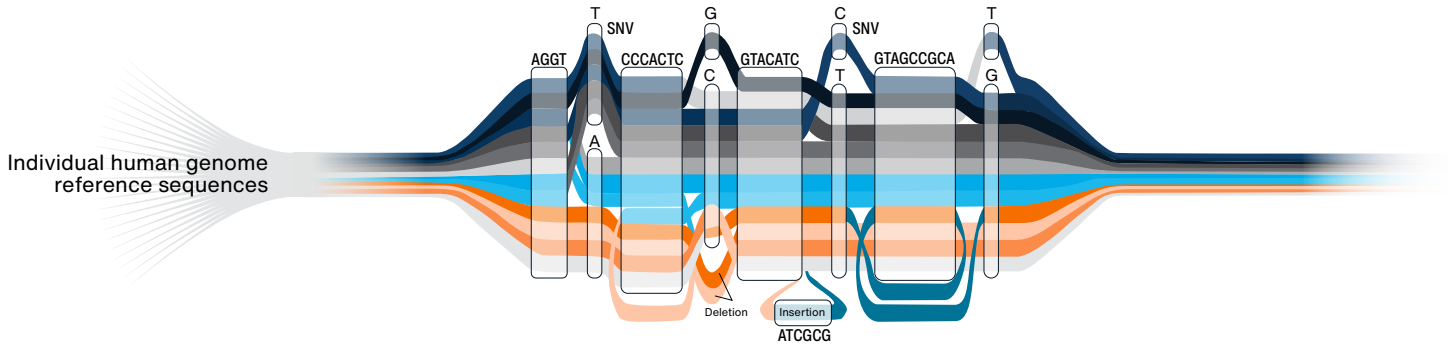

Figure 2. The pangenome incorporates genomes from multiple individuals of varying ancestral backgrounds to more accurately represent global human variation in sequence and structure. Specifically, pangenome enables characterization of common benign structural variants such as deletions, duplications and insertions of large DNA segments as depicted by the looping colored segments.

*Adapted from National Human Genome Research Institute**

genome, which originates largely from individuals of European ancestry, contains technical gaps, and misses substantial genomic variation, especially structural variants. Through better representation of global diversity, pangenome mitigates historical reference biases and helps improve diagnostic accuracy across racial and ethnic backgrounds. This pangenome-enabled approach increases diagnostic confidence across diverse patient populations by improving variant detection, reducing reference bias, and expanding coverage into previously "dark" regions of the genome.

Enhancing Resolution with Integrated RNA Analysis

The integration of RNA sequencing into Ambry's genome sequencing test enhances diagnostic precision and expands the ability to uncover critical insights for rare disease patients and their families. RNA analysis provides essential insights into the functional impact of DNA variants, offering a more nuanced understanding of their potential contributions to human disease (Table 2). Ambry's GenomeReveal™ enables the addition of RNA testing for eligible variants into genome sequence analysis, improving the ability to classify variants that are predicted to impact the splicing process. Data from Ambry's ExomeReveal® and published literature show that RNA analysis increases diagnostic yield by ~2-3% and it is estimated that ~one-third of variants eligible for RNA analysis are noncoding variants that can only be detected by genome sequencing.[10]

RNA Testing Provides Additional Insight for Multiple Variant Types
Establish pathogenicity for intronic VUS
Increase confidence in likely pathogenic and pathogenic classifications of intronic variants
Correct misclassification of canonical variants where observed impact ≠ predicted impact
Uncover clinically significant synonymous variants
Establish pathogenicity for missense variants with splice impact

Table 2: The table outlines the various applications of RNA testing in genetic variant analysis, enhancing the accuracy and comprehensiveness of genetic variant interpretation.

Patient for Life™: Proactive Reanalysis for a Lifetime of Answers

Ambry's reanalysis approach is an unparalleled model that elevates the standard of care for all patients. Patient for Life is the first laboratory-initiated, cohort-level reanalysis program of its kind. This innovative service proactively reviews patient data for new diagnostic insights based on advances in gene-disease validity and variant classification and leads to generation of reclassification reports that are shared directly with ordering clinicians. New gene-disease relationships continue to be the primary contributor of diagnostic yield in unresolved cases, further amplifying the value of genome sequencing as novel genes and associations are discovered through proactive reanalysis.[11]

*https://www.genome.gov/genetics-glossary/Pangenome

Since its launch, Ambry's Patient for Life program has delivered clinically meaningful reclassifications to 5% of patients who previously received negative or uncertain exome sequencing results (Figure 3).[12] Patient for Life also helps close gaps inherent in clinician-initiated reanalysis workflows by ensuring equitable access to updated and accurate genetic testing results for all patients.[13]

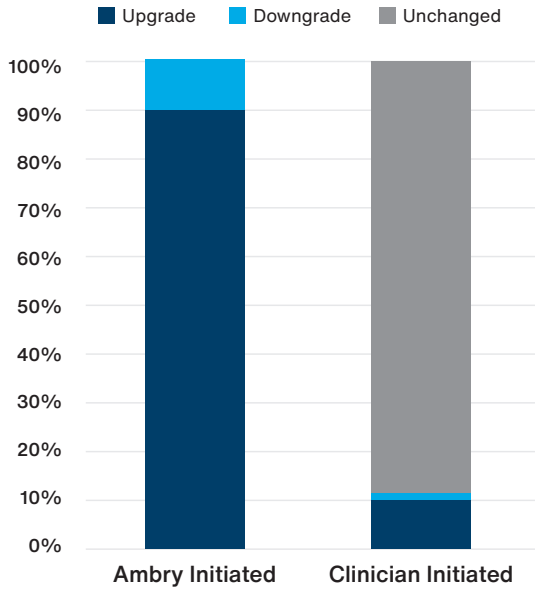

Figure 3. Ambry-initiated reanalysis leads to increased clinically relevant reclassifications.

Patients who undergo genome sequencing at Ambry are automatically enrolled in the Patient for Life reanalysis program, where their data is continuously reviewed to provide updates to genetic results, ensuring that new genomic discoveries directly enhance their clinical care.

Conclusion

Genome sequencing enables comprehensive detection of a wide spectrum of variant types in both coding and non-coding regions. Ambry further harnesses the power of genome sequencing by incorporating RNA sequencing to clarify functional impact, pangenome mapping to improve variant detection and coverage in diverse populations, and Patient for Life continuous reanalysis to proactively update results as new data emerges. By integrating these advancements, Ambry Genetics provides an equitable and comprehensive genomic solution designed to uncover more answers for patients today and as scientific discovery continues to evolve.

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