Real-World Evidence Demonstrating Why Genome Sequencing Should Be Recommended as the First-Tier Genetic Test

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BACKGROUND

- Individuals with suspected rare genetic diseases often undergo lengthy diagnostic journeys. Typically, they endure multiple clinical evaluations and an exhaustive series of laboratory tests.

- As one of the most comprehensive genetic tests that interrogates single nucleotide variants (SNV), copy number variants (CNV), mitochondrial variants, repeat expansion, and structural variants in one single assay, genome sequencing (GS) has long been expected to increase the diagnostic yield and end the diagnostic odyssey. Despite the clear technical superiority, the full clinical utility of GS has yet to be determined.

- We systematically evaluated 2100 clinical GS index cases performed in our laboratory since 2017 to explore the diagnostic yield of GS as first-tier and follow-up tests.

- Highlights of the genome sequencing performed at RVTY:
  - Mean coverage of 40x throughout the entire Genome;
  - Complete coverage of >99% of the exome, including over 5,400 disease-associated genes;
  - Reliable detection of intragenic deletions and duplications in clinically relevant genes as well as large-scale CNV events and structural rearrangement events;
  - Include the analysis of the mitochondrial genome
  - Include repeat expansion disorder screening of more than 30 genes associated with intellectual disability and movement disorders (since 2020)
  - Include SMN1 copy number screening for Spinal Muscular Atrophy (SMA) (since 2020)

DEFINITION OF DIAGNOSTIC OUTCOME

The diagnostic yield was determined based on the association of the reportable variants with the patient's phenotype and clinical manifestations. At the case level, the index cases were classified into four categories.

- **Definitive diagnostic cases:** Cases with 1 pathogenic or likely pathogenic (P/LP) variant in the genes for autosomal dominant (AD), X-linked disorders, or the mitochondrial genome associated with the submitted phenotype, and the cases with homozygous or compound heterozygous P/LP variants in the genes associated with autosomal recessive (AR) disorders which explain the phenotype observed.

- **Assumed diagnostic cases:** Cases with 1 P/LP variant and 1 variant of uncertain significance (VUS) in AR disorder genes with or without phase information, cases with confirmed de novo VUS variants in AD or X-linked disorder genes; and cases with homozygous or two VUS variants in trans in AR disorder genes. The VUS variants included here are highly suspected to be disease-causing but cannot be classified as P/LP based on current ACMG guidelines without further functional or segregation analysis.

- **Cases with VUS findings:** Cases with a single VUS in AD genes or two VUSs in AR genes that could explain the patient's phenotype and are consistent with the inheritance pattern.

- **Cases with apparently negative cases:** Cases with no reportable variants or only 1 P/LP/VUS variant in the AR diseases that might potentially contribute to the disease.

Both definitive diagnostic cases and assumed diagnostic cases were considered for the diagnostic yield.
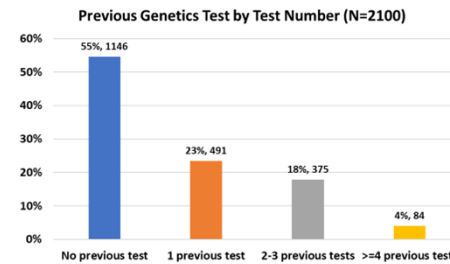
RESULTS

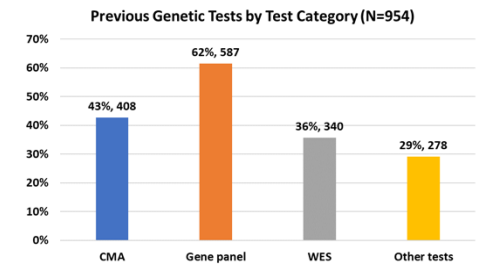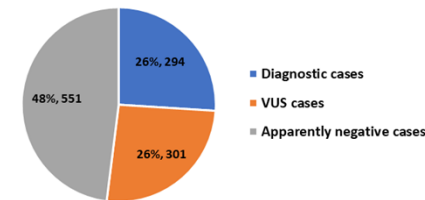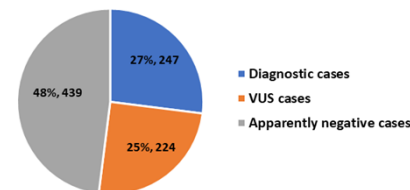**Figure 1** GS cases with previous genetics test by test numbers

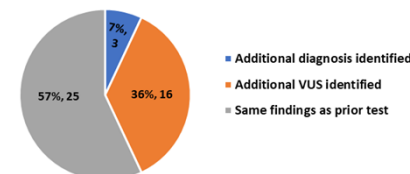**Figure 2** GS cases with previous genetic test by test category
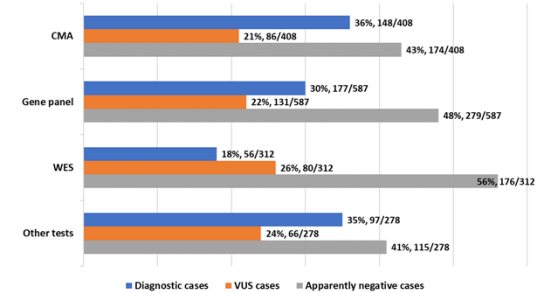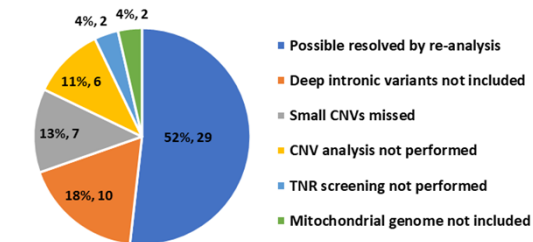

Figure 3. Diagnostic yield of GS as a first-tier test verse as a follow-up test and the Key reasons contributing to the missed diagnosis by prior ES test.

CONCLUSION

- We present the largest-to-date GS data from a clinically heterogeneous cohort in a single clinical laboratory.

- Our data demonstrated that GS should be recommended as the first-tier genetic test to shorten the diagnostic odyssey. For patients with previous inconclusive genetic test results, including ES, GS should still be considered to further explore the genetic etiology of their conditions.