Genome Sequencing

ORDER NAME

Cerner: Whole Genome Sequencing,Proband
WGSPRO, WGSCOM-Sunquest codes for label generation only (no charge)

The healthcare provider is also required to complete a Genome Sequence Analysis Proband requisition form and a signed Genome Sequence Analysis Family Member requisition form for each participant as well as submit the pedigree and relevant clinical notes to GenomicsLab@upmc.edu

BACKGROUND

Genome sequence analysis (GS) is one of the most comprehensive clinical tests to identify rare genetic alterations and can be used as either a first-tier diagnostic approach or following extensive prior molecular testing in patients who remain undiagnosed. GS evaluates the entire genome, including both protein-coding and non-coding regions, and enables detections of both small sequence variants and copy number variants. GS also includes the analysis of the mitochondrial genome, and screening for repeat expansion disorders associated with the following genes: *AR, ARX, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8OS, C9orf72, CACNA1A, CNBP, DMPK, FMR1, FXN, HOXD13, PABPN1, PHOX2B, TBP.* Optional reporting of secondary findings is available. These findings may be unrelated to the primary indication for testing but could have important implications for medical management. A curated list of such genes is defined by the American College of Medical Genetics and Genomics (ACMG), focusing on conditions for which established interventions may reduce morbidity and/or mortality. Trio testing, including both biological parents, is strongly recommended to improve variant interpretation by enabling inheritance-based analysis.

INDICATIONS FOR TESTING

- Provides hybridization- free/PCR-free sequencing of the human genome, enabling coverage of intergenic regions and detection of variant types not assessed by traditional exome sequencing.
- The diagnostic rate can yield as high as 30-57% when strict clinical criteria are applied.
- First-tier test substantially reduces the time to diagnosis at only 25%-50% of the cost of traditional testing.
- An early and accurate molecular diagnosis can lead to optimal care and dramatic prognostic improvements for patients and their families.
- Identifying a disease-causing variant in a patient provides preconception and prenatal options for at-risk family members of reproductive age.

RESULTS

Pathogenic, likely pathogenic, and variants of uncertain significance related or possibly related to the patient's symptoms are reported. Only pathogenic and likely pathogenic variants are reported for ACMG secondary findings.

Positive: A pathogenic or likely pathogenic variant(s) were found which would either explain the patient's symptoms or increases the risk to develop a disease. The healthcare provider can use the result to guide the patient's medical management. Family members can be tested for the variant to determine their risk for disease and/or reproductive risk.

Negative: No clinically significant variants were found in the test. This does not rule out the possibility of variants in other genes or variants that are not detectable in this assay. Risk may still be increased based on the family history. The healthcare provider will discuss these risks and develop a screening plan based on the patient's personal risk factors. The healthcare provider may also discuss more testing either now or in the future.

Variant of Uncertain Significance (VUS): A variant was detected, however, it is uncertain whether this variant is the cause of a patient's symptoms since current information about the variant is limited. The result is not clinically actionable. Medical management should be based on personal and family history.

METHOD

Genomic DNA is fragmented, adaptors are ligated to the fragment ends, and libraries are sequenced on Illumina next generation sequencing (NGS) systems using 2×150 bp paired-end reads at the High Throughput Genomics Core. The average mean sequencing coverage across the nuclear genome is at least 30X. Bi-directional sequence reads are assembled and aligned to the GRCh38 human genome reference. Sequencing variants including single nucleotide substitutions (SNVs), small deletions, small insertions, small indels, copy number variants (CNVs), and short tandem repeat (STR) variants are detected using the Illumina DRAGEN Bio-IT Platform. Variants are called at a minimum of 8X coverage and a variant allele fraction of ≥20%. Mitochondrial reads are mapped to the NC_012920.1 reference, with an average mean coverage of at least 1000X, and mitochondrial variants are called at allele fractions ≥5%. Variants are annotated using commercially available software and are interpreted and classified according to ACMG/AMP standards and guidelines. Reportable variants that do not meet laboratory quality criteria are confirmed using Sanger sequencing, ddPCR, long-range PCR, breakpoint PCR, MLPA, or another validated method prior to reporting. Variants classified as likely benign or benign are not confirmed or reported.

LIMITATIONS

This assay is designed to detect single nucleotide substitutions (SNVs), small deletions (≤20 bp), small insertions (≤10 bp), small indels, and copy number variants (CNVs) greater than 1 kb in size. Some genomic regions have inherent complex sequence features such as homology, pseudogene content, or high GC composition that may yield suboptimal data and increase the likelihood of missed variants across variant types. Detection of CNVs smaller than 1 kb is limited and not validated for routine clinical reporting; however, potential sub-kilobase CNV findings may be further evaluated at the discretion of the laboratory director. CNV detection sensitivity may be reduced in regions with variable coverage, poor mappability, high GC content, or repetitive sequences. Mitochondrial CNVs are not assessed. CNV analysis does not detect balanced chromosomal alterations including reciprocal or Robertsonian translocations, balanced inversions, or complex genomic rearrangements. This test does not detect methylation abnormalities. The assay is not specifically designed to detect mosaicism; however, potential mosaic findings may be further evaluated at the discretion of the laboratory director. Although molecular tests are highly accurate, rare diagnostic errors may occur.

A total of 18 repeat expansion disorders are evaluated from the WGS data (*AR, ARX, ATN1, ATXN1,ATXN2, ATXN3, ATXN7, ATXN8OS, C9orf72, CACNA1A, CNBP, DMPK, FMR1, FXN, HOXD13, PABPN1, PHOX2B, TBP*). NGS-based repeat expansion screening provides a definitive rule-out only when two distinct non-expanded alleles are identified. When a single non-expanded allele is detected, either true homozygosity or the presence of an undetected expansion beyond assay limits is possible. Follow-up testing using traditional methods (e.g., triplet-primed PCR or Southern blot) is required when clinically indicated. The upper detection limit for expanded alleles using NGS is not fully established; therefore, expanded alleles suggested by NGS will require independent confirmatory testing for accurate sizing.

Variants related to phenotype are reported based on analysis of clinical information provided by the ordering provider. There may be variants of uncertain significance present in the sample with partial overlap to some of the given phenotypic information which were not determined to be relevant enough for reporting. A list of all variants identified in this individual is available upon request. The classification and interpretation of all variants identified in

this assay reflect the current state of the laboratory's scientific understanding at the time this report was issued. Variant classification and interpretation may change for a variety of reasons, including, but not limited to, improvements to classification techniques, availability of additional scientific information and observation of a variant in more patients.

SPECIMEN REQUIREMENTS

- Peripheral blood collected in EDTA, 3 ml, consult director for smaller volumes
- Previously extracted DNA (Concentration >14.3 ng/µl, volume >20ul, minimum of 2 ug -DNA from blood;1.1 ug gDNA from saliva)
- Saliva provided in Oragene DNA (OGD-500) collection kits accepted for relatives only
- Fibroblasts

TURNAROUND TIME

Six weeks from the receipt of all family members who choose to participate, which is no later than 30 days from receipt of the patient's sample.

CPT CODE

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