GenomeSeqDx: Clinical Genome Sequencing

DESCRIPTION

GenomeSeqDx, or clinical genome sequencing (GS) can be used to identify the underlying molecular basis of a genetic disorder in an affected individual with:

- One or more congenital anomalies[1]
- Neurodevelopmental disorder including developmental delay[1]
- Unexplained epilepsy[2-3]
- A phenotype suggestive of a genetic etiology that does not correspond to a specific condition for which genetic testing is available[4-9]
- A suspected genetic condition that has a high degree of genetic heterogeneity[10]

Clinical genome sequencing simultaneously evaluates both the protein-coding and non-coding regions of the human nuclear genome, allowing for the potential detection of characterized/pathogenic variants in regions that are not assessed by exome sequencing (ES). The protein-coding regions represent ~20,000 genes and account for approximately 2% of all human genetic material.[11] The non-coding regions include promoter, intronic, and untranslated regions. While much of the data generated from sequencing the genome is not well understood at this time, GS may provide more reliable coverage of the exonic regions.[12-13] GS has lower depth of coverage on average compared to ES, but more positions are covered to adequate depth for accurate variant calling.

For GenomeSeqDx, an individual's nuclear genome sequence is filtered against published reference sequences, and compared to other sequenced family members, population databases, control sequences, and the GeneDx internal database to reveal potentially reportable variants. The affected individual's sequence is then evaluated using gene-phenotype associations, in conjunction with multiple resources including gnomAD, OMIM, PubMed, and ClinVar.[14] Sequence changes of interest are interpreted according to the American College of Medical Genetics and Genomics (ACMG) guidelines.[15] A report is issued with clinically relevant variants, which may include sequencing nucleotide variants (SNV), copy number variants (CNV) greater than 1 kb, uniparental disomy (when parents are submitted), homozygous loss of exon 8 in the *SMN1* gene, and repeat expansions in select genes that are associated with the patient's reported phenotype.

Genome sequencing identifies a causal variant in approximately 30-57% of probands, with a higher yield when both parents are included and have strict clinical inclusion criteria.[4-10]

For GenomeSeqDx nuclear genome analysis, an affected individual's clinical records and prior genetic testing results submitted at the time of test activation will be reviewed prior to analysis. The proband's analysis includes evaluation of variants that are identified to be *de novo* (when both parents submitted), compound heterozygous (when both parents submitted), homozygous, heterozygous and X-linked. In addition, analysis takes into consideration family structure, reported phenotype, and provided clinical and/or differential diagnosis. This is a comprehensive analysis of a very large number of genes with phenotype-driven reporting; therefore, reported results are focused on pathogenic and likely pathogenic variants in genes related to the clinical information provided. Less frequently, variants of uncertain significance in candidate and differential diagnosis genes are reported.

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RESULT REPORTING

Nuclear genome sequence analysis is performed on the proband and parental samples, and/or additional relatives as needed, when submitted together for analysis. A report will be issued only for the affected proband in the family. A separate report will not be issued for parents or other family members who may also have submitted a specimen for the purpose of allowing better interpretation of the results from the affected individual. If additional reports are requested for other family members, additional fees may apply.

The report issued for the affected proband may contain variations in genes previously implicated in a human disease similar to the clinical presentation of the affected individual or in genes hypothesized to be related to the cause of the disease (candidate genes or genes of unknown significance). Variants in candidate genes may be reported based upon limited published evidence (including case report(s)) and/or internal data, such as observations of previous cases with similar phenotypes and types of variants in the same gene. In addition, other evidence such as gene function, tissue of expression, and phenotype of model organisms with alterations in the gene also may be used as evidence to support reporting a variant in a candidate gene.

ACMG Secondary Findings

ACMG recommends that secondary findings identified in a specific subset of genes associated with medically actionable, inherited disorders be reported for all probands undergoing exome or genome sequencing. Please refer to the latest version of the ACMG recommendations for reporting of secondary findings in clinical exome and genome sequencing for complete details of the genes and associated genetic disorders. Secondary findings will be included for all exome or genome sequencing reports, unless a family opts-out of receiving this information on the Informed Consent as part of the test requisition form. The status of any secondary finding(s) reported for the affected individual will be provided for all relatives included as part of the proband's test; GeneDx does not conduct an independent evaluation of secondary findings in relatives. Relatives have the ability to opt-out of receiving secondary findings. Secondary findings will be confirmed by an alternate test method, as indicated.

Sequence Analysis and Deletion Testing of the Mitochondrial Genome

The mitochondrial genome sequencing and deletion test results are issued in a separate report. For more information on the mitochondrial genome sequencing and deletion testing, please visit our "Mito Genome Sequencing & Deletion Testing" page on our website.

TEST METHODS

Genomic DNA from the submitted specimen is sequenced with paired-end reads on an Illumina platform. Average mean sequencing coverage for the proband is at least 40x across the genome, with a minimum threshold of 30x for any individual sample. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19.

Data are filtered and analyzed to identify sequence variants, repeat expansions of select genes, homozygous loss of *SMN1* exon 8 (Spinal Muscular Atrophy), and most deletions and duplications greater than 1 kb in size. Screening for disease associated repeat expansions is included for the following genes: *AR, ARX* (PA1 only)*, ATN1, ATXN1, ATXN2, ATXN3, ATXN7, ATXN8/ATXN8OS, C9orf72, CACNA1A, CNBP, DMPK, FMR1*

(with methylation status for alleles with >150 repeats*), FXN, HOXD13, PABPN1,* and *PHOX2B* (see table below for more details). Unless otherwise reported, the proband is considered screen negative for these non-sequencing variants; however, if a high clinical suspicion of any of these disorders is present, gene/disease specific diagnostic testing should be considered.

Reportable variants include pathogenic and likely pathogenic variants related to the reported phenotype. Variants of uncertain significance, likely benign and benign variants, and pathogenic or likely pathogenic variants not associated with the reported phenotype, if present, are not routinely reported. Reported variants are confirmed by an appropriate orthogonal method in the proband and, if submitted, in selected relatives as necessary.

Sequence variants are reported according to the Human Genome Variation Society (HGVS) guidelines. Copy number variants are reported based on the probe coordinates, the coordinates of the exons involved, or precise breakpoints when known. Repeat expansions are reported with an accuracy of +/- 2 repeats for alleles that contain up to 150 total nucleotides (e.g., 50 triplet repeats or 25 hexanucleotide repeats) and +/- 5 repeats for alleles that contain >150 nucleotides. The exact number of repeats cannot be determined for very large alleles, which will be reported as "greater than" a gene-specific threshold. Available evidence for variant classification may change over time and reported variant(s) may be reclassified according to the ACMG/AMP Standards and Guidelines[15], which may lead to issuing a revised report.

LIMITATIONS

The GenomeSeqDx test attempts to evaluate the complete coding and non-coding regions of the genome. However, it is not technically possible to uniquely resolve and align the entire genome at present due to homology and other structural complexities in some regions. GenomeSeqDx is limited in the types of variants that are detected and reported including nucleotide repeat expansion (with the exception of those noted in this document) and some structural variants. It is anticipated that approximately 97% of the coding region of an affected individual's nuclear genome (i.e., the exome) will be assessed with the GenomeSeqDx test at 15x coverage.

The available scientific knowledge about the function of all genes in the human genome is incomplete at this time. It is possible that the GenomeSeqDx test may identify the presence of a genetic variant in the genomic sequence of an affected individual, but it will not be recognized as causative for the affected individual's disorder due to insufficient knowledge about the variant or the gene and its function. With the exception of ACMG Secondary findings, pathogenic or likely pathogenic variants that are identified as part of the analysis, but not known or expected to be associated with the reported phenotype at the time of analysis will not be routinely reported. Reanalysis of the nuclear genome data is available upon request by the healthcare provider to incorporate updated clinical information and/or newly emerging gene and variant information. Updates to the classification of a sequence variant may be accessed through ClinVar (www.clinvar.com). Even if this test identifies the underlying genetic cause of a disorder in an affected individual, it is possible that such a diagnosis will not permit an accurate prediction of the prognosis or severity of the disease. While there is a possibility that identifying the genetic cause may help direct management and treatment of the disease, it is also possible that this knowledge will not change management or treatment.

TEST INFORMATION SHEET

Genes Screened for Repeat Expansions by GenomeSeqDx:

Reference Ranges of Repeat Expansions for Genes Evaluated by GenomeSeqDx						
Gene	**Positive**	**Incomplete Penetrance**	**Premutation**	**Intermediate**	**Uncertain Significance**	**Normal**
*AR**	38 or greater	35-37				34 or less
ARX (PA1)	23 or greater				19-22	18 or less
*ATN1**	48 or greater	36-47	21-35			20 or less
*ATXN1**	47 or greater				36-46	35 or less
*ATXN2**	35 or greater (AD)	33-34			31-32	30 or less
*ATXN3**	60 or greater				45-59	44 or less
*ATXN7**	37 or greater	34-36	28-33		20-27	19 or less
*ATXN8/ATXN8OS**	80 or greater				51-79	50 or less
*CACNA1A**	20 or greater		19			18 or less
*C9orf72**	61 or greater			25-60		24 or less
*CNBP**	75 or greater			27-74		26 or less
DMPK	50 or greater		35-49			34 or less
FMR1	201 or greater		55-200	45-54		44 or less
FXN	66 or greater	44-65	34-43			33 or less
HOXD13	22 or greater				16-21	9-15
*PABPN1**	12-17 (AD) 11 (AR)					10
PHOX2B	26 or greater (AD)	24-25			21-33	20 or less

AD=autosomal dominant
AR=autosomal recessive
*=typically adult onset
Not Applicable

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