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, and control sequences to reveal potentially reportable variants. The affected individual's sequence is then evaluated using a phenotype-driven approach that includes Human Phenotype Ontology and Human Gene Mutation Database 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 guidelines.[15]

Genome sequencing identifies a causal variant in approximately 30-57% of probands, with a higher yield for cases that specifically include both parents and have strict clinical inclusion criteria.[4-10]

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 unaffected 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 affected family members, additional fees may apply.

The report issued for the affected proband in the family 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), based upon the function, tissue of expression, and phenotype of model organisms with alterations in the gene. Variants in candidate genes

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may also be reported based on internal data, such as observations of previous cases with similar phenotypes and types of variations in the same gene.

ACMG Secondary Findings

The American College of Medical Genetics and Genomics (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 when needed.

The mitochondrial genome sequencing and deletion test results are issued in a separate report.

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 30x across the genome. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19.

A custom-developed analysis tool is used to filter and analyze data for the identification of variants. Reported clinically significant variants, are confirmed, if necessary, by an appropriate orthogonal method in the proband and, if submitted, in selected relatives. 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.

GenomeSeqDx also includes screening for several non-sequencing variants commonly related to human disease. This test can identify uniparental disomy (UPD) when parents are submitted, homozygous loss of exon 8 (formerly exon 7) in the *SMN1* gene (spinal muscular atrophy), and expansion of the poly-nucleotide repeat regions in the *FMR1* (FMR1-related disorders) and *DMPK* (myotonic dystrophy, type 1) genes. If detected, expansions of greater than 54 CGG repeats in the *FMR1* gene and greater than 49 CTG repeats in the *DMPK* gene are confirmed via an appropriate orthogonal method and reported. 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.

For GenomeSeqDx nuclear genome analysis, an affected individual's submitted clinical records and prior genetic testing results 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 phenotype-driven test of a very large number of genes; 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.

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 above) 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. 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.

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