XomeDx®: Clinical Exome Sequencing

DESCRIPTION

XomeDx®, or exome sequencing (ES), can be used to identify the underlying molecular basis of a genetic disorder in an affected individual. It is recommended that ES be considered for individuals with:

- One or more congenital anomalies[1]
- Neurodevelopmental disorders including developmental delay, intellectual disability, and autism spectrum disorders[1-2]
- Unexplained epilepsy[3]
- A phenotype suggestive of a genetic etiology that does not correspond to a specific condition for which genetic testing is available[4]
- A suspected genetic condition that has a high degree of genetic heterogeneity[4]
- A suspected genetic condition for which other available genetic testing options did not identify a diagnosis[4]

The *XomeDx*® test targets the protein-coding regions (exons) of ~20,000 genes, which account for approximately ~2% of the human genome.[5] These targeted regions are captured, sequenced using next-generation sequencing, 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.[6] Sequence changes of interest are interpreted according to the American College of Medical Genetics and Genomics guidelines.[7] Exome sequencing identifies a causal variant in 33-38% of cases, and yields the highest diagnostic rate when both biological parents or family members are included.[8-10]

RESULT REPORTING

Exome sequence analysis is performed on the affected individual (i.e., the proband) and, when submitted together for analysis, biological parental samples, and/or additional biological relatives as needed. A report will be issued only for the proband. 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 for the affected individual. If additional reports are requested for other affected family members, additional fees will apply.

The report issued for the proband may contain variations in genes previously implicated in a human disease that has clinical features similar to those in 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 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.

TEST METHODS

Using genomic DNA from the submitted specimen(s), DNA is enriched for the complete coding regions and splice site junctions for most genes of the human genome using a proprietary capture system developed by GeneDx for next-generation sequencing. The enriched targets are simultaneously sequenced with paired-end reads on an Illumina platform. Bi-directional sequence reads are assembled and aligned to reference sequences based on NCBI RefSeq transcripts and human genome build GRCh37/UCSC hg19. Using a custom-developed analysis tool, data are filtered and analyzed to identify sequence variants and copy number variants (CNVs) involving three or more exons.[11] 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.

An affected individual's submitted clinical records and previous genetic testing results are reviewed prior to analysis. The proband's analysis includes evaluation of variants that are identified to be *de novo* (when both biological parents submitted), compound heterozygous (when both biological 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 *XomeDx*® test attempts to evaluate the most important regions of the majority of the ~20,000 genes in the human genome. However, it is not technically possible to capture and sequence the entire exome at present. It is anticipated that approximately 98% of the targeted region of an affected individual's exome will be assessed with the *XomeDx*® test at a minimum of 10x coverage, the minimum sequencing read depth necessary to detect a variant. Across the exome, the average depth of coverage is 100-120x. The test report will include case-specific exome coverage. There may be some genes or portions of genes that are not amenable to capture, sequencing, and alignment. Additionally, certain types of sequence variations are difficult to identify or may not be possible to identify using ES, such as repeat expansions. In some instances, CNV detection may not be possible using the available sequencing data, and if so, this will be indicated in the final report. Average read depth statistics for the *XomeDx*® test are as follows:

Read Depth	10x	20x	30x	40x	50x
Mean Percent of Target Covered	98%	98%	97%	95%	91%

The available scientific knowledge about the function of all genes in the human genome is incomplete at this time. It is possible that the *XomeDx*® test may identify the presence of a genetic variant in the exome 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 data is available upon request by the health care provider to incorporate updated clinical information and/or newly emerging gene and variant information. Even if the *XomeDx*® 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.

REFERENCES:

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