XomeDx: Whole Exome Sequencing
XomeDxPlus: Whole Exome Sequencing with Mitochondrial Genome Sequencing / Deletion Testing

Description:
XomeDx, or whole exome sequencing (WES), can be used to identify the underlying molecular basis of a genetic disorder in an affected individual. The XomeDx test is different from other types of genetic diagnostic tests in terms of the number of genes that are sequenced simultaneously. WES can be used to identify the molecular basis of a genetic disorder in individuals:

- With a genetically heterogeneous disease, as pathogenic findings could be present in many different genes
- With a long list of differential diagnoses
- With an atypical presentation of a genetic disorder
- Who have exhausted other currently available genetic testing options

The XomeDx test targets the protein-coding regions of the human genome, which represents ~20,000 genes and accounts for approximately ~2% of all human genetic material (Bamshad et al., 2011). These targeted regions of an individual's genes, called exons, are captured and sequenced using massively parallel sequencing. An individual's sequence is then compared to published reference sequences, other individuals from the affected individual's family, and control individuals, and phenotype-driven gene lists are generated using Human Phenotype Ontology and HGMD gene-phenotype associations. Additional resources such as 1000 Genomes database, NHLBI Exome Sequencing Project, ExAC, OMIM, PubMed, and Clinvar are used to evaluate genes and detect sequence changes of interest, which are then interpreted according to the American College of Medical Genetics and Genomics guidelines (Retterer et al., 2016; Richards et al., 2015). The XomeDx test is most effective when other family members (both biological parents, if available) are included in the analysis of the affected individual's exome sequence. Past evaluation of the diagnostic yield of WES testing has shown that it leads to a diagnosis in 23-26% of cases; however, the diagnostic yield increases when additional family members are tested (Yang et al., 2013; Lee et al., 2014; Retterer et al., 2016). In an analysis of 3,040 XomeDx cases, a definitive result was reported in 23.6% of cases in which only the proband was analyzed, and increased to 31.0% when the proband and two family members were analyzed by WES (Retterer et al., 2016).

XomeDxPlus is a combined test including whole exome sequencing with mitochondrial genome sequencing and deletion testing. XomeDxPlus is best suited for individuals with clinical features suggesting a mitochondrial disorder. For more information on the

mitochondrial genome sequencing and deletion component of the XomeDxPlus testing, please visit our neurology/mitochondrial genetics page on our website.

Result Reporting:
Whole exome sequence analysis is performed on the proband and parental samples, and/or additional relatives as needed, when submitted together for analysis. A single XomeDx or XomeDxPlus report will be issued on the affected individual in the family. A separate report will not be issued for unaffected parents or other unaffected 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 will apply.

The XomeDx or XomeDxPlus report issued for the affected individual in the family will contain variations in genes previously implicated in a human disease similar to 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 XomeDx 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, known and/or expected pathogenic variants, identified in a specific subset of genes associated with medically actionable, inherited disorders be reported for all probands undergoing whole exome sequencing. Please refer to the latest version of the ACMG Recommendations for Reporting of Secondary Findings in Clinical Exome and Genome Sequencing Report for complete details of the genes and associated genetic disorders. Secondary findings will be included for all XomeDx and XomeDxPlus reports, unless a family opts-out of receiving this information on the Informed Consent and Authorization Form as part of the XomeDx Test Requisition Form. The status for any secondary finding(s) reported for the affected individual will be provided for all relatives tested by XomeDx or XomeDxPlus; 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.

Test Methods:
An affected individual's clinical records and prior genetic testing results will be reviewed prior to analysis. Using genomic DNA from the submitted specimen(s), the exonic regions and flanking splice junctions of the genome are sequenced by massively parallel (NextGen)

sequencing on an Illumina sequencing system with 100bp or greater paired-end reads. Reads are aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis tool (Xome Analyzer). Capillary sequencing or another appropriate method is used to confirm all potentially pathogenic variants identified in the individual and relative samples, if submitted. Sequence alterations are reported according to the Human Genome Variation Society (HGVS) nomenclature guidelines.

Analysis of XomeDx for the proband 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 to relevant analysis based on the family structure and reported phenotype. Variants are reported based on the clinical information provided.

For **XomeDxPlus,** whole exome sequencing (as described above) is performed concurrently with mitochondrial genome sequencing and deletion testing. Using genomic DNA, the entire mitochondrial genome is amplified by long-range PCR and sequenced using a novel solid-state sequencing-by-synthesis process that allows sequencing a large number of amplicons in parallel (Bennett, 2004). DNA sequences are assembled and compared to the published mitochondrial genome reference sequences for analysis. The presence of any disease-associated sequence variant is confirmed by conventional dideoxy sequence analysis or other methods. A reference library of more than 6000 samples from different ethnic groups and online databases for mtDNA variations will be used to evaluate variants of unknown clinical significance identified in the mitochondrial genome. Full mitochondrial genome sequencing will be performed on the proband only. If a maternal sample is provided at the time of the proband's sample submission, maternal samples will undergo Sanger sequencing to identify if reported mitochondrial findings are inherited, however maternal level of heteroplasmy will not be evaluated. Carrier testing for maternal relatives can be ordered separately.

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 95% of the targeted region of an affected individual's exome will be assessed with the XomeDx test at 10x coverage, while >98% of the target region will be covered at a minimum of 1x. 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 using WES, including repeat expansions and copy number variants.

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:

1. Bamshad et al. (2011) Nature Reviews. Genetics 12 (11):745-55 (PMID: 21946919)
2. Yang et al. (2013) N. Engl. J. Med. 369 (16):1502-11 (PMID: 24088041)
3. Lee et al. (2014) Jama 312 (18):1880-7 (PMID: 25326637)
4. Retterer et al. (2016) Genet. Med. 18 (7):696-704 (PMID: 26633542)
5. Richards et al. (2015) Genetics In Medicine 17 (5):405-24 (PMID: 25741868)
6. Bennett et al. (2004) Pharmacogenomics 5 (4):433-8 (PMID: 15165179)