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

Description:
XomeDx, or exome sequencing (ES), 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. ES 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 GnomAD NHLBI Exome Sequencing Project, 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. Several large studies have demonstrated that exome sequencing identifies a causal variant in 25-30% of cases, with a higher yield for cases that specifically include other family members (Retterer et al., 2016; Farwell et al., 2015; Lee et al., 2014; Yang et al., 2014).

XomeDxPlus is a combined test including 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:
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 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), 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 and copy number variants (Retterer et al., 2015). Reported clinically significant variants are confirmed by an appropriate orthogonal method in the proband and, if submitted, in selected relatives as necessary. Sequence and copy number variants are reported according to the Human Genome Variation Society (HGVS) or International System for Human Cytogenetic Nomenclature (ISCN) guidelines, respectively. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. Likely benign and benign variants, if present, are not routinely reported.

The analysis 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, 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.

For **XomeDxPlus,** exome sequencing (as described above) is performed concurrently with mitochondrial genome sequencing and deletion testing. Using genomic DNA from the submitted sample, the entire mitochondrial genome is amplified and sequenced using Next Generation sequencing. DNA sequence is assembled and analyzed in comparison with the revised Cambridge Reference Sequence (rCRS GeneBank number NC_012920) and the reported variants listed in the MITOMAP database (http://www.mitomap.org). Next-generation sequencing may not detect large-scale mtDNA deletions present at 5% heteroplasmy or lower or mtDNA point variants present at 1.5% heteroplasmy or lower. Alternative sequencing or other detection methods may be used to analyze or confirm mtDNA variants. Reportable variants include pathogenic variants, likely pathogenic variants and variants of uncertain significance. 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, reportable sequence variants present at 10% or greater heteroplasmy will be evaluated in the maternal sample by PCR-amplification of the relevant portion(s) of the mitochondrial genome from genomic DNA (Sanger sequencing). For analysis performed by Sanger sequencing, levels of mutant heteroplasmy 25% or lower may not be detected. Targeted testing of identified mtDNA variants 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 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 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 using ES, such as repeat expansions. Average read depth statistics for the XomeDx test are as follows:

Read Depth	10x	20x	30x	40x	50x
Mean Percent of Target Covered	98%	97%	94%	90%	83%

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. Retterer et al. (2016) Genet. Med. 18 (7):696-704 (PMID: 26633542)
3. Richards et al. (2015) Genetics In Medicine 17 (5):405-24 (PMID: 25741868)
4. Farwell et al. (2015) Genet. Med. 17 (7):578-86 (PMID: 25356970)
5. Lee et al. (2014) Jama 312 (18):1880-7 (PMID: 25326637)
6. Yang et al. (2014) JAMA 312 (18):1870-9 (PMID: 25326635)
7. Kalia et al. (2017) Genet. Med.19 (2):249-255 (PMID: 27854360)
8. Green et al. (2013) Genet. Med.15 (7):565-74 (PMID: 23788249)